CHAPTER 4

Results and Discussions

4.1 Experiment 1: The study of optimum parameters for Vacuum Cooling Process of baby cos lettuce vacuum cooling.

4.1.1 The vacuum cooling parameter for vacuum cooling of baby cos lettuce

Different process parameters (final pressure and reserving time) were used to operate the precooling process. The total of 55 cycles were investigated in this study. The optimum process parameters for the produces, which had initial of 20-25 ºC were at final pressure of 6.0 mbar with 25-30 min reserving time. The produces which initial temperature of 18-20 ºC were at final pressure of 6.0 mbar with 20-25 min reserving time For produces that had initial temperature of 16-18 ºC, the optimum process parameters were at final pressure of 6.5 mbar with 15-20 min reserving time, with the previous three process parameter sets, the final temperature of the produce would be successfully pulled down to 4±1 ºC (Table 4.2). Produce with high initial temperature required low final pressure setting for enhanced the evaporation of water vapor and removed more field heat from produce. When water evaporated, it needs to absorb latent heat, which must be supplied from the product or from the surroundings in order to maintain higher energy level of molecular movement at gaseous state that consequently produce were refrigerated. In addition, longer reserving time were recommend to produce which high initial temperature in order to encourage the better heat transfer between produce and cold air in the vacuum chamber.

Table 4.1 Vacuum cooling parameters for vacuum cooling process of baby cos lettuce

No.	Final pressure	Reservin g time	Produce core temp (°C)		Weigh t loss	Cycle time	Cooling rate	Electric cost
	(mbar)	(min)	Initial	Final	(%)	(min)	(°C/min)	kWh
1	5	10	18.60	7.10	0.75	20	0.58	3.19
2	5.5	10	18.30	4.50	1.25	27	0.51	2.81
3	5.5	10	17.07	4.90	1.40	20	0.61	2.63
4	5.5	10	19.40	6.95	1.16	22	0.57	2.63
5	5.5	15	17.30	3.00	1.75	27	0.53	4.13
6	5.8	12	13.40	1.80	1.89	20	0.58	1.97
7	6	20	16.75	5.50	2.77	35	0.32	1.97
8	6	10	16.60	6.60	1.47	20	0.50	2.25
9	6	15	16.87	3.30	1.49	29	0.47	5.44
10	6	15	18.80	4.90	1.51	30	0.46	4.41
11	6	10	17.07	5.30	1.33	22	0.54	3.38
12	6	18	17.87	3.53	2.47	27	0.53	3.75
13	6	10	17.70	6.90	1.49	24	0.45	4.22
14	6	20	17.00	6.10	2.65	37	0.29	3.38
15	6	25	26.13	4.60	2.60	35	0.62	4.20
16	6	25	18.20	1.87	2.33	34	0.48	4.97
17	6	15	15.00	11.30	1.24	26	0.14	3.00
18	6	10	19.35	9.60	1.48	20	0.49	1.78
19	6	17	22.00	4.75	2.56	29	0.59	4.13
20	6.2	14	15.35	5.40	2.78	22	0.45	3.09
21	6.4	20	17.42	3.67	2.82	27	0.51	4.03
22	6.3	19	17.40	4.90	2.64	27	0.46	3.56
23	6.5	25	16.93	5.70	2.72	35	0.32	5.63
24	6.5	20	18.07	6.50	2.08	34	0.34	4.78
25	6.5	20	18.10	4.70	2.22	30	0.45	5.06
26	6.5	15	18.83	6.80	1.88	26	0.46	2.81
27	6.5	18	18.33	7.40	2.83	28	0.39	4.03
28	6.5	25	18.50	7.60	3.66	37	0.29	4.97
29	6.5	20	18.85	7.00	2.46	31	0.38	2.63
30	6.5	15	18.05	4.00	3.89	23	0.61	3.38

Table 4.1 Vacuum cooling parameters for vacuum cooling process of baby cos

lettuce (continued)

No.	Final pressure	Reserving time	Produce core temp (°C)		Weight loss	Cycle time	Cooling rate	Electric cost
	(mbar)	(min)	Initial	Final	(%)	(min)	(°C/min)	kWh
31	6.7	28	17.44	3.47	2.53	39	0.36	5.44
32	6.8	25	19.00	7.03	3.16	37	0.32	5.25
33	7	20	18.23	5.00	2.86	32	0.41	4.41
34	7	20	17.93	6.70	1.93	32	0.35	3.75
35	7	20	16.85	5.80	1.91	32	0.35	4.22
36	7	25	21.63	6.50	1.57	35	0.43	5.44
37	7	30	17.80	7.30	3.26	38	0.28	4.97
38	7.2	24	16.30	5.23	2.05	33	0.34	5.16
39	7.5	30	15.40	6.17	2.40	40	0.23	5.91
40	7.5	25	17.40	6.70	1.65	36	0.30	5.16
41	7.5	22	18.00	6.10	2.19	33	0.36	4.50
42	8	25	17.37	5.20	1.97	34	0.36	5.06
43	8	28	16.30	6.50	1.79	38	0.26	5.44
44	8	10	18.85	11.63	0.68	22	0.33	3.19
45	8	20	19.55	8.80	1.84	29	0.37	3.38
46	8.5	22	19.85	8.03	1.32	34	0.35	5.44
47	8.5	29	15.20	7.20	2.34	37	0.22	4.88
48	9	10	18.56	10.70	1.16	23	0.34	3.09
49	9	25	23.10	8.65	2.24	38	0.38	4.88
50	9	22	17.50	8.25	1.62	36	0.26	5.16
51	9	18	18.48	8.30	1.34	27	0.38	4.13
52	9.5	30	16.20	8.20	2.41	42	0.19	5.91
53	9.3	24	21.60	11.50	2.59	35	0.29	5.16
54	9.4	29	20.12	9.30	2.71	41	0.26	5.44
55	10	30	17.30	10.90	2.47	44	0.15	5.34

Table 4.2 Recommendation parameters setting for vacuum cooling of baby cos lettuce

Initial product temperature (°C)	Final pressure (mbar)	Reserving time (min)
16.0-18.0	6.5	15-20
18.0-20.0	6.0	20-25
20.0-25.0	6.0	25-30

4.1.2 The relationship of pressure and temperature during vacuum cooling process

The principle of vacuum cooling is the relationship between atmospheric pressure and the boiling point of water which pressure, volume and temperature are the important factors in vacuum cooling process. Generally, the boiling point changes as a function of saturation pressure especially for vacuum cooling, atmospheric pressure is the predominant effect on the boiling of water (2). Thereby the boiling point will be reduced following by reduced atmospheric pressure. At atmospheric pressure (1014 mbar), the boiling temperature of water is 100°C. If the ambient pressure is reduced to 23.37 mbar, the water boiling temperature will be 20°C and at 6.09 mbar, it will be 0°C corresponding to the lower pressure operation of vacuum cooler should limit at 6.0 mbar in order to avoid freezing injury that will occur in perishable produce during vacuum cooling. Moreover, using low pressure under 6.0 mbar may cause the high cost production due to the amount of extra work by the vacuum pump. In some case, such as vacuum cooling of head lettuce can reduced the pressure to 5.07 mbar (29).

The process of vacuum pump in vacuum cooling operation can explain by two steps. The first step initiate when the chamber door is closed and the pump is started. In the meantime, the water vapor saturation pressure is reduced to lower than the atmospheric pressure and continues until reach the “flash point” (Figure 4.1) where the atmospheric pressure has been reduced to the water vapor saturation pressure depending on the initial produce temperature. At the same time the water vapor start evaporate from produce due to boiling begins under low pressure condition. After that, the second step happens at saturation until the desired final produce temperature is reached depended on the reserving time setting (4).

Vacuum cooling of baby cos lettuce with the parameter setting at final pressure 6.0 mbar and reserving time 25 minute showed that during the first step of vacuum

cooling process, the produce temperature was closely with the chamber temperature and remain constant around 18 °C while the chamber pressure was reduced from atmospheric pressure (974 mbar) until the second step started at 10 min, the beginning of "flash point" was occurred and the measured produce temperature dropped suddenly from 17.6 to 6.2 °C within 30 min and continued convection heat transfer which slowly decrease to 5.2 °C within 35 min, in the meanwhile the chamber temperature has shown the similar trend (Figure 4.1). The most vaporization of water occurred around the produce surface shortly after the flash point, through it is possible occurred in the intercellular spaces. The results shown the high level of the relative humidity in the chamber up to 88.6% during the first step of vacuum cooling until reached flash point and after the evaporation of water the relative humidity was rapidly decrease to 49.4% (Figure 4.2) In term of weight loss, lettuce cooled approximately 12 °C for each 1 % loss in weight (62). The experiment results showed that there is 2.33% of weight loss appeared after vacuum cooling process. In some case, vacuum cooling may cause weight loss up to 5% of its moisture during the vacuum cycle. There are some recommended to reduce moisture losses by wetting the produce before or during the vacuum process or apply the water spray above the produce but should considerate about water sanitation and desired packaging which could be effect on produce qualities. The cooling rate is depended on several factor including the surface area and volume ratio, the rate of vacuum which could create in the chamber and the rate of heat conduction of the produce (29). However, continued holding at low pressure was necessary to obtain a desirable final temperature for the produce (4).

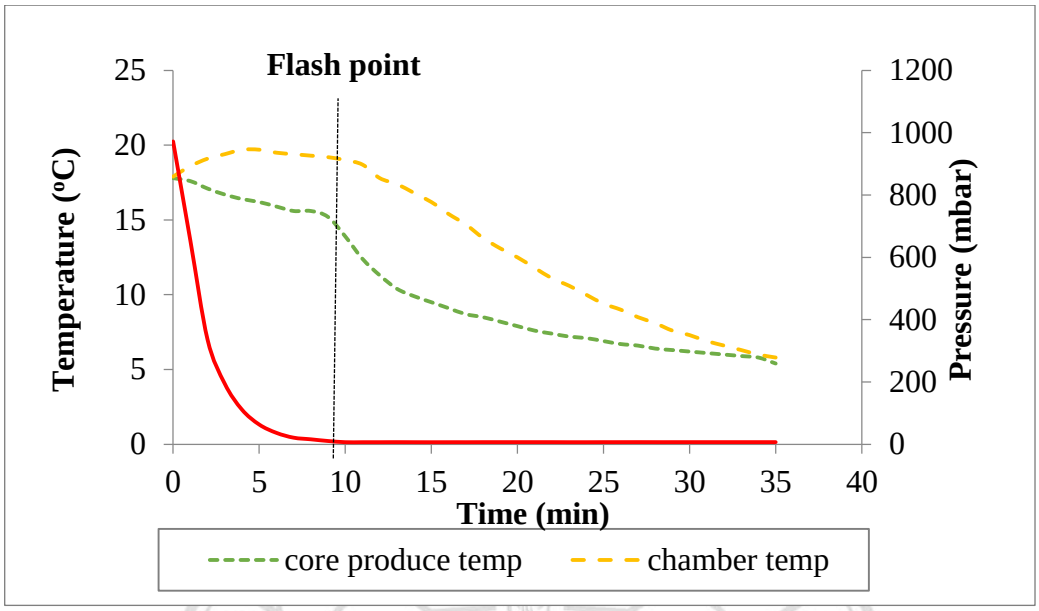

Figure 4.1 Relationship of pressure and temperature during vacuum cooling process of baby cos lettuce

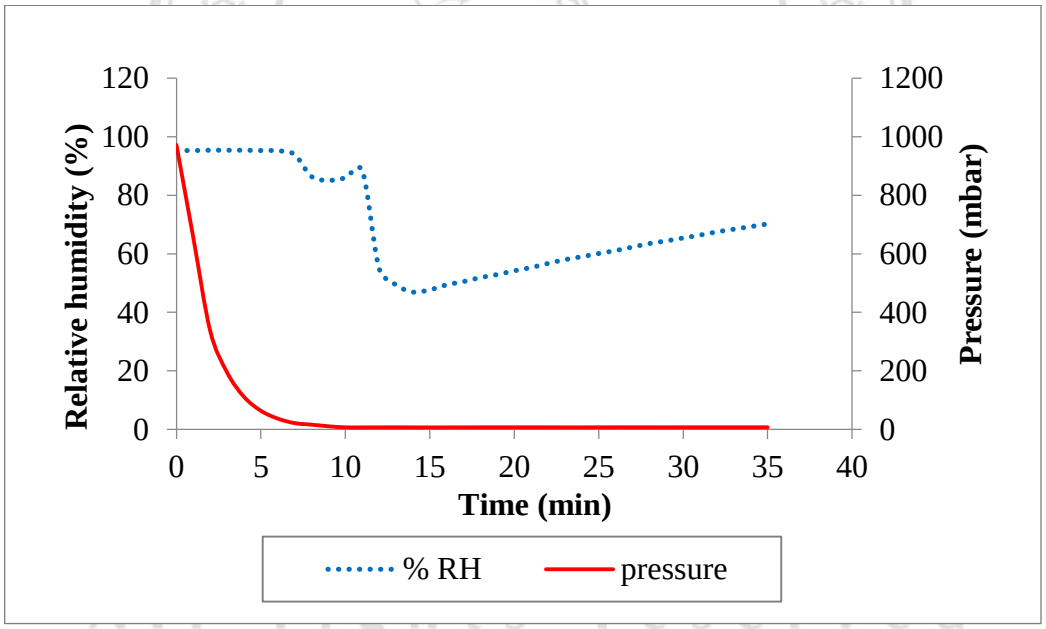

Figure 4.2 Relationship of pressure and temperature during vacuum cooling process of baby cos lettuce

4.2 Experiment 2: Prediction of Baby cos lettuce final temperature and weight loss percentage using Artificial Neural Network (ANNs) and Multiple linear regression (MLR)

4.2.1 The optimization of number of input for vacuum cooling process prediction using Artificial Neuron Network (ANNs)

Vacuum cooling parameters data form the experiment 1 were used to train with an artificial neural network based on back propagation to predict final temperature and weight loss percentage of vacuum cooling process for baby cos lettuce. The experiment data were trained by Levenberg-Marquardt algorithm which divided to training data 70% (36 data), testing 20% (8 data) and validation 20% (8 data). First, the number of input variables was optimized to improve the predictability of ANNs. Input parameters consisted of 5 parameters, which were final pressure, reserving time, initial temperature, chamber temperature and initial weight of sample and output results were final temperature and weight loss percentage. The number of input parameters used was varied from 5, 4 and 3 inputs. Moreover, the number of neurons in one-hidden-layer was varied from 1 to 30 for each inputs variable. The performances of various ANNs was determined from higher R^2_{adjust} and lower RMSE, MRE% and MAE. Results showed that, the high performance predictability of hidden layer neurons for final temperature output of 5, 4 and 3 inputs were 22, 17 and 13 neurons, respectively as shown in Figure 4.3-4.8. The best prediction performance was the prediction using 4 inputs parameter with 17 neurons in hidden layer which represented the highest R^2_{adjust} (0.885) and lowest RMSE, MRE% and MAE (0.076, 10.28 and 0.504, respectively) (Table 4.3). Similarly, the results of weight loss prediction demonstrated that the optimum neurons in hidden layer for 5, 4 and 3 input parameters was 17, 19 and 20 neurons, respectively (Figure 4.9-4.14) and the best fit model for weight loss prediction was the prediction using 3 input parameters with 20 neurons in hidden layer which yielded highest R^2_{adjust} (0.897) and lowest RMSE, MRE% and MAE (0.078, 7.680 and 0.133, respectively) as shown in Table 4.4.

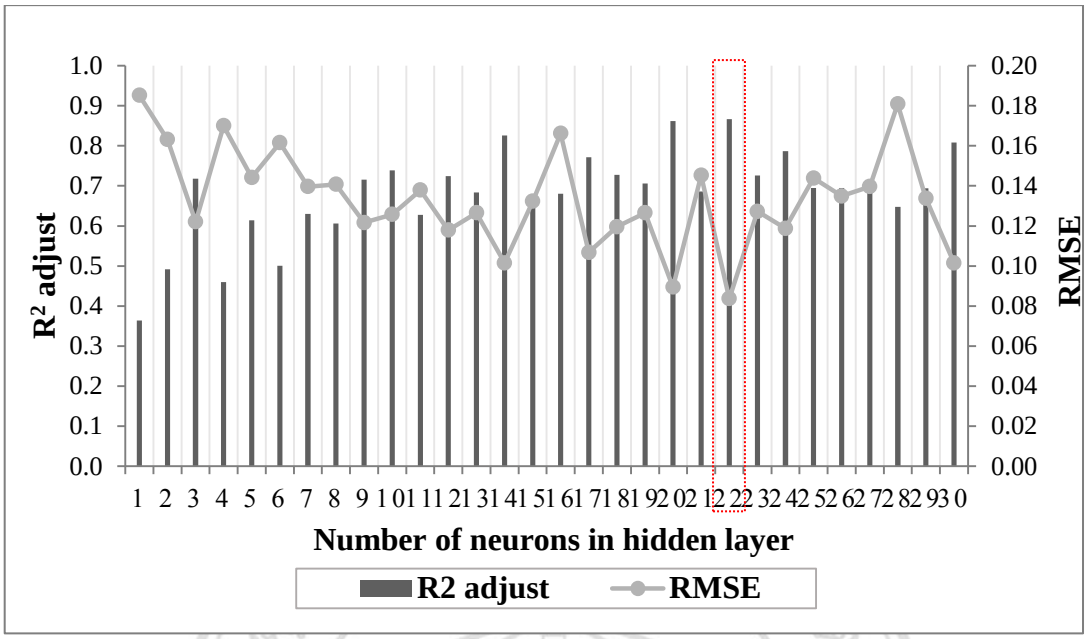

Figure 4.3 Comparison of ANNs performance for final temperature prediction with 5 input parameters and 1-30 neurons in hidden layer

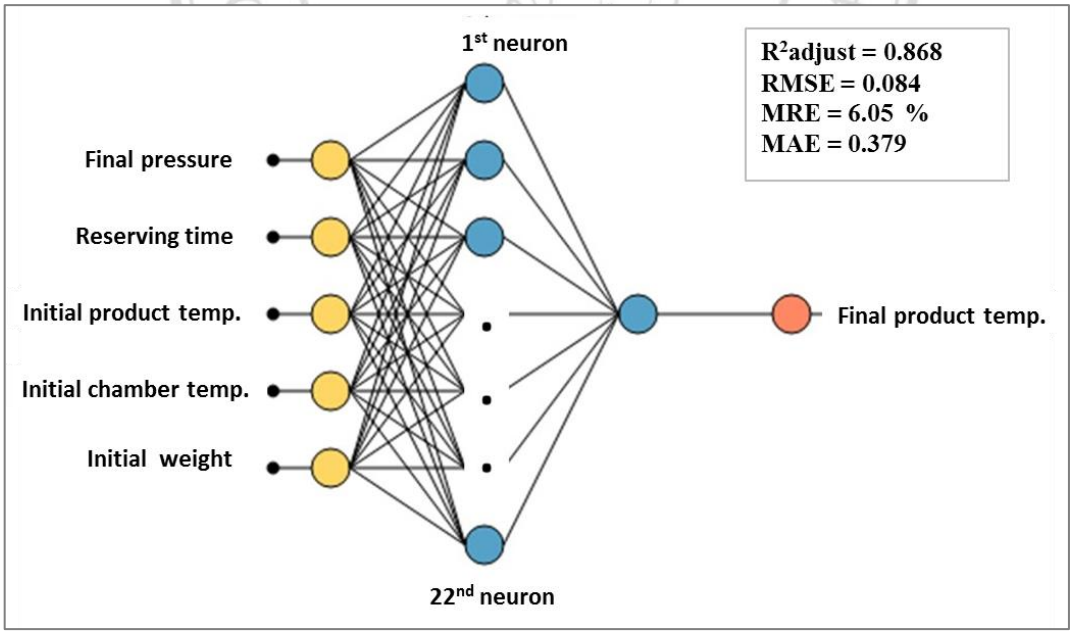

Figure 4.4 The optimum number of hidden layer neurons for final temperature prediction with 5 input parameters

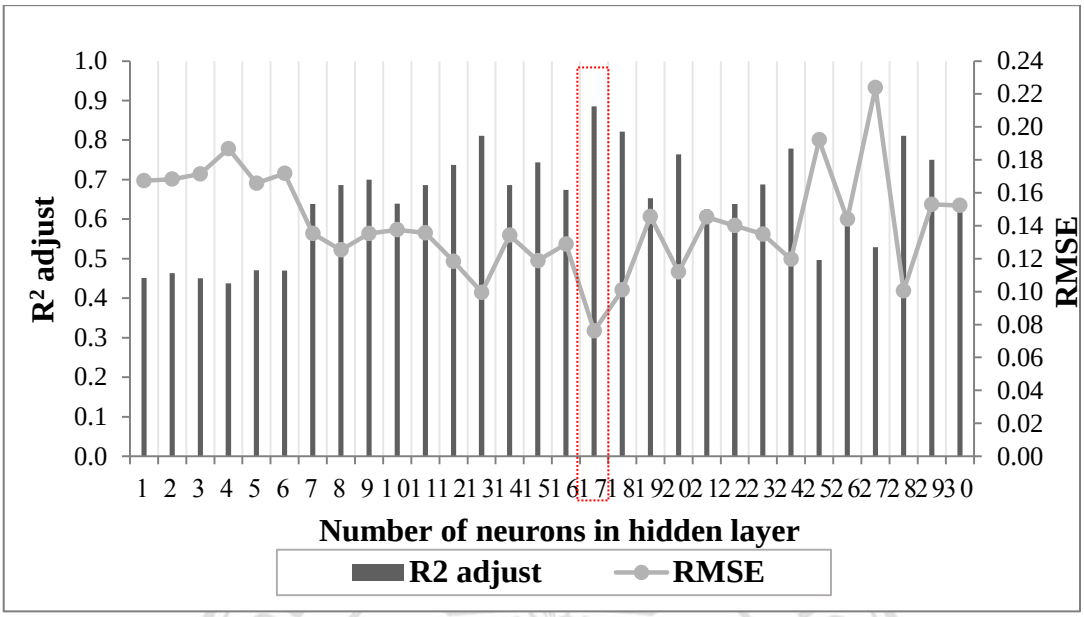

Figure 4.5 Comparison of ANNs performance for final temperature prediction with 4 input parameters and 1-30 neurons in hidden layer

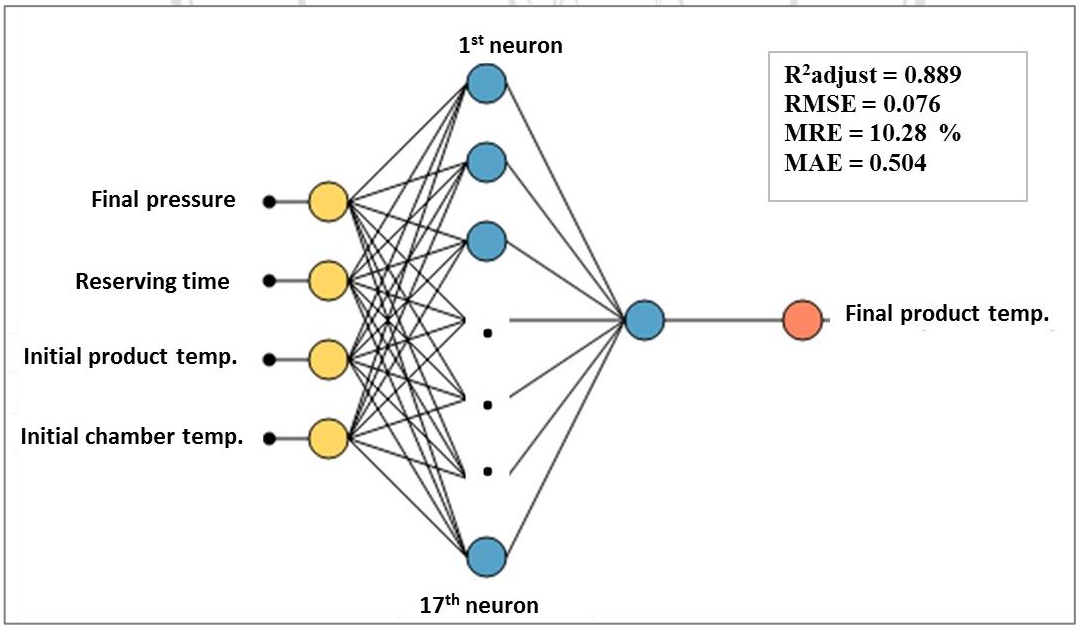

Figure 4.6 The optimum number of hidden layer neurons for final temperature prediction with 4 input parameters

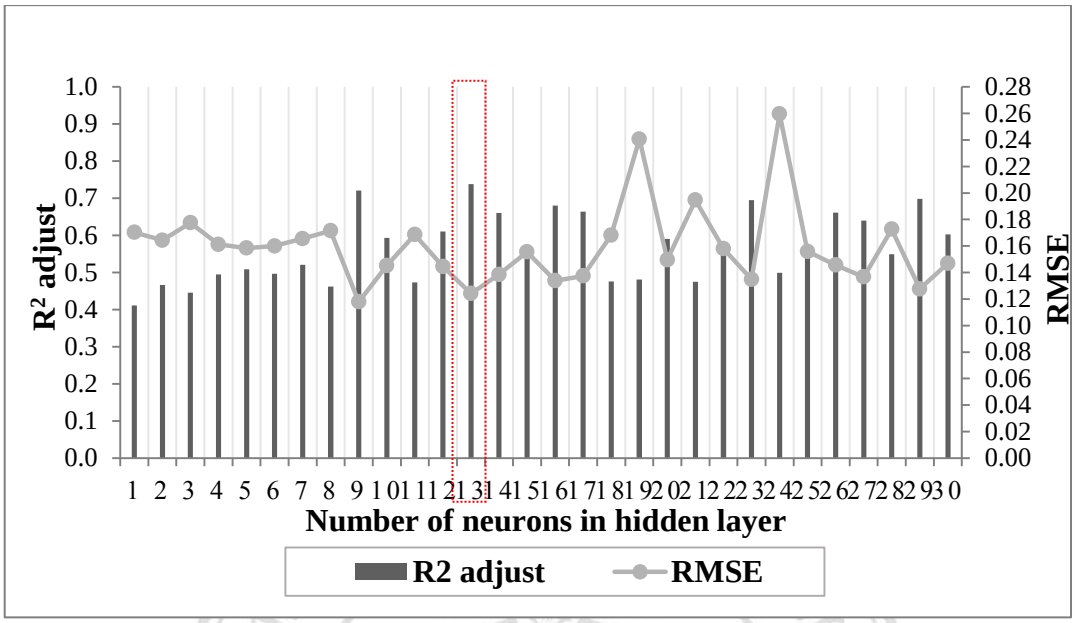

Figure 4.7 Comparison of ANNs performance for final temperature prediction with 3 input parameters and 1-30 neurons in hidden layer

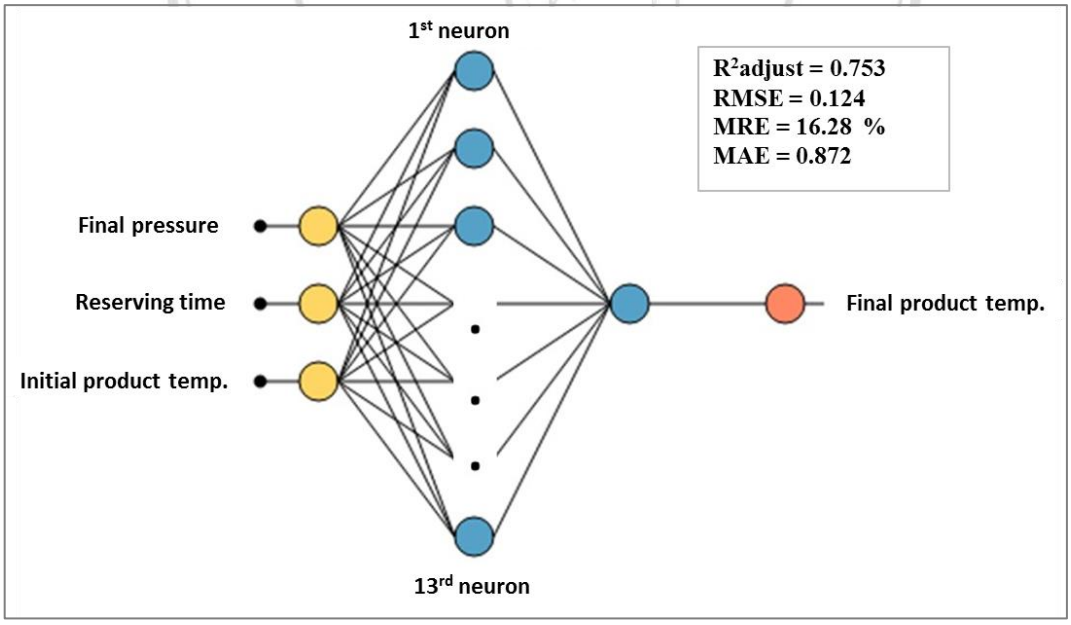

Figure 4.8 The optimum number of hidden layer neurons for final temperature prediction with 3 input parameters

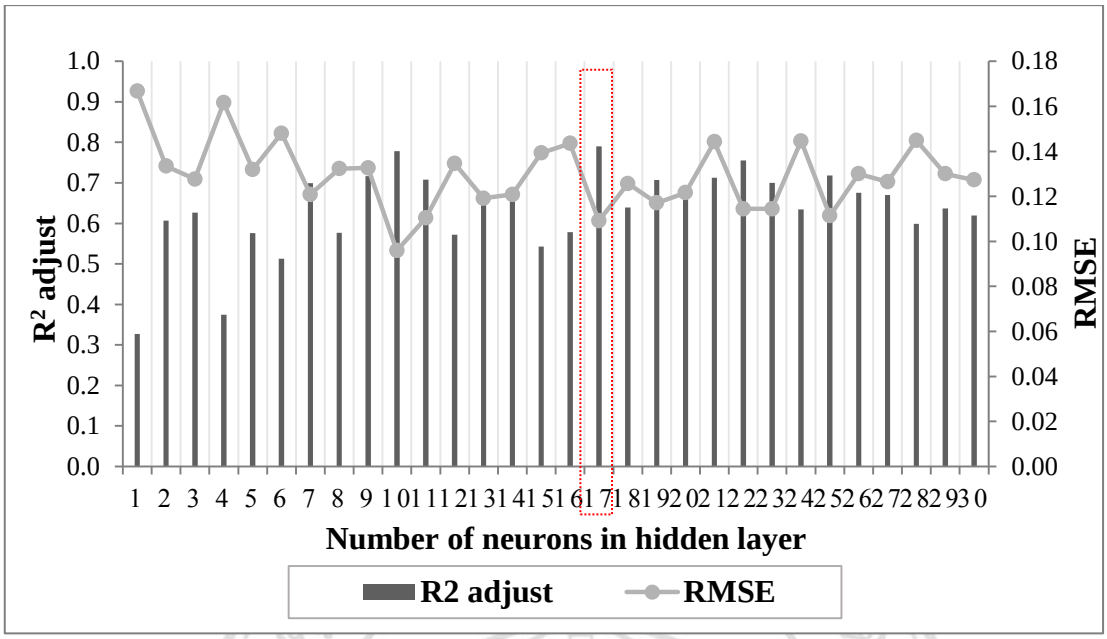

Figure 4.9 Comparison of ANNs performance for weight loss percentage prediction with 5 input parameters and 1-30 neurons in hidden layer

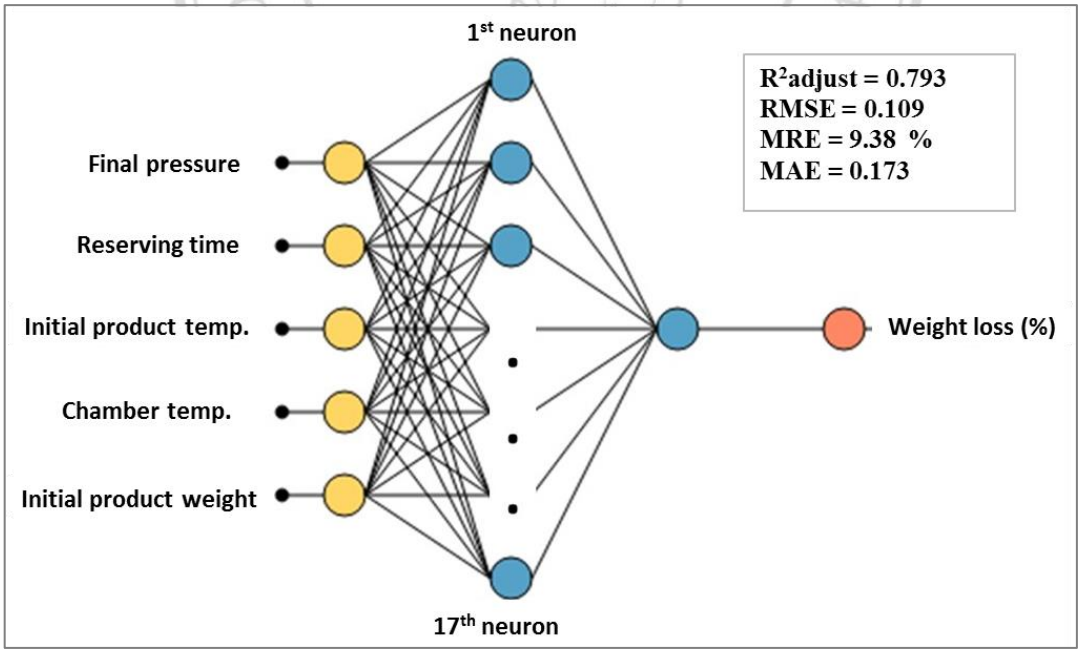

Figure 4.10 The optimum number of hidden layer neurons for weight loss percentage prediction with 5 input parameters

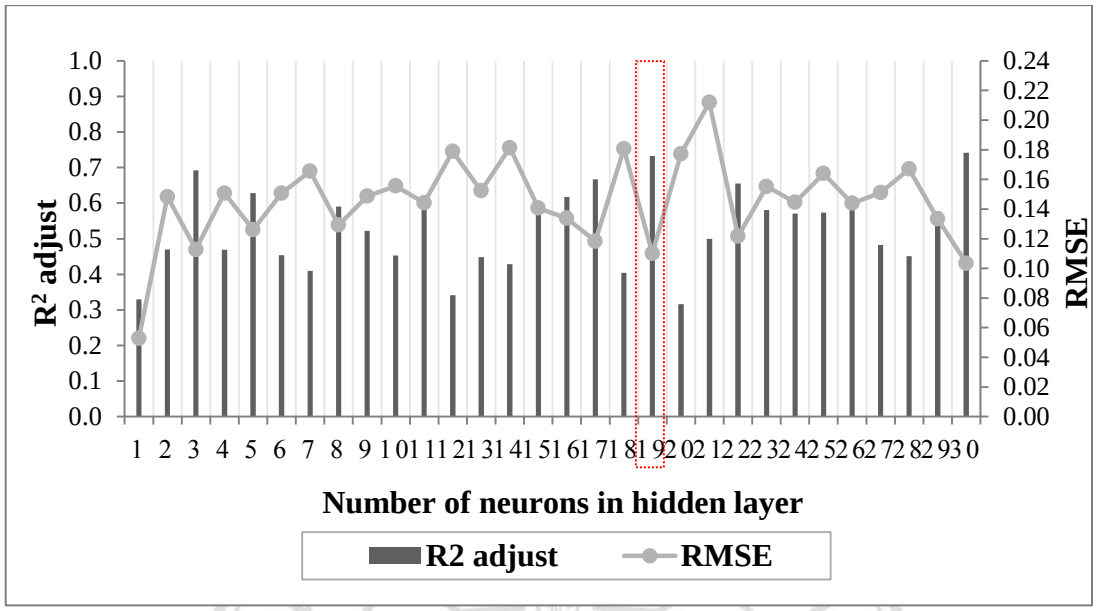

Figure 4.11 Comparison of ANNs performance for weight loss percentage prediction with 4 input parameters and 1-30 neurons in hidden layer

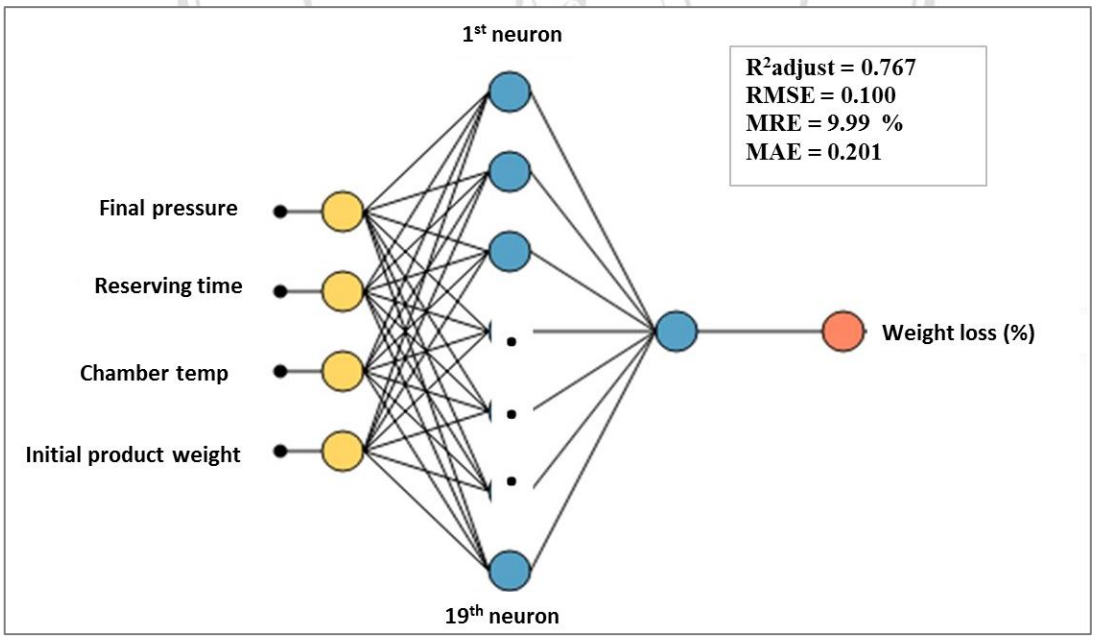

Figure 4.12 The optimum number of hidden layer neurons for weight loss percentage prediction with 4 input parameters

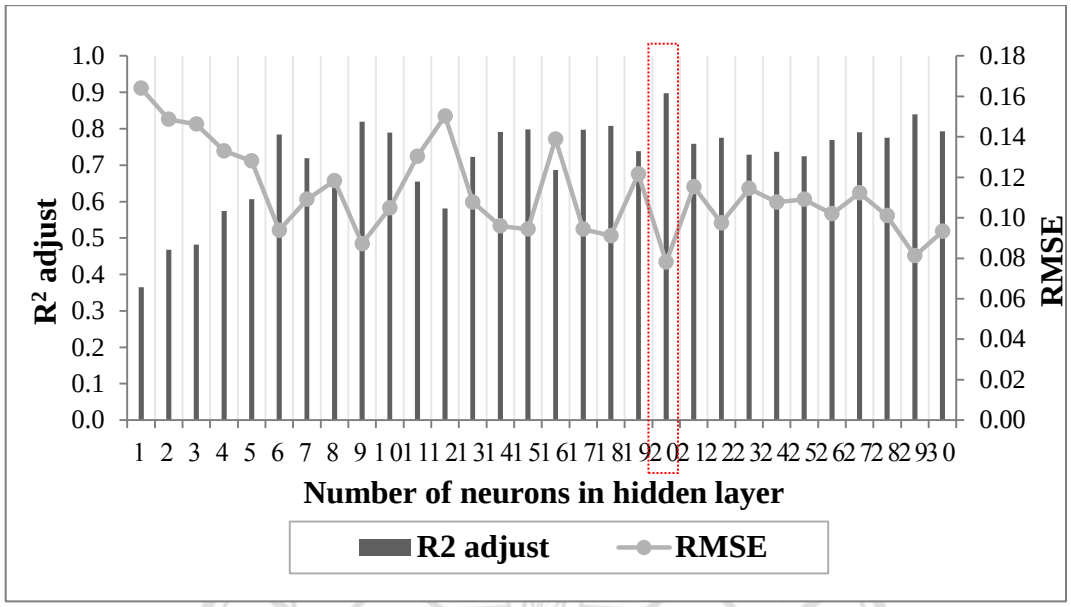

Figure 4.13 Comparison of ANNs performance for weight loss percentage prediction with 3 input parameters and 1-30 neurons in hidden layer

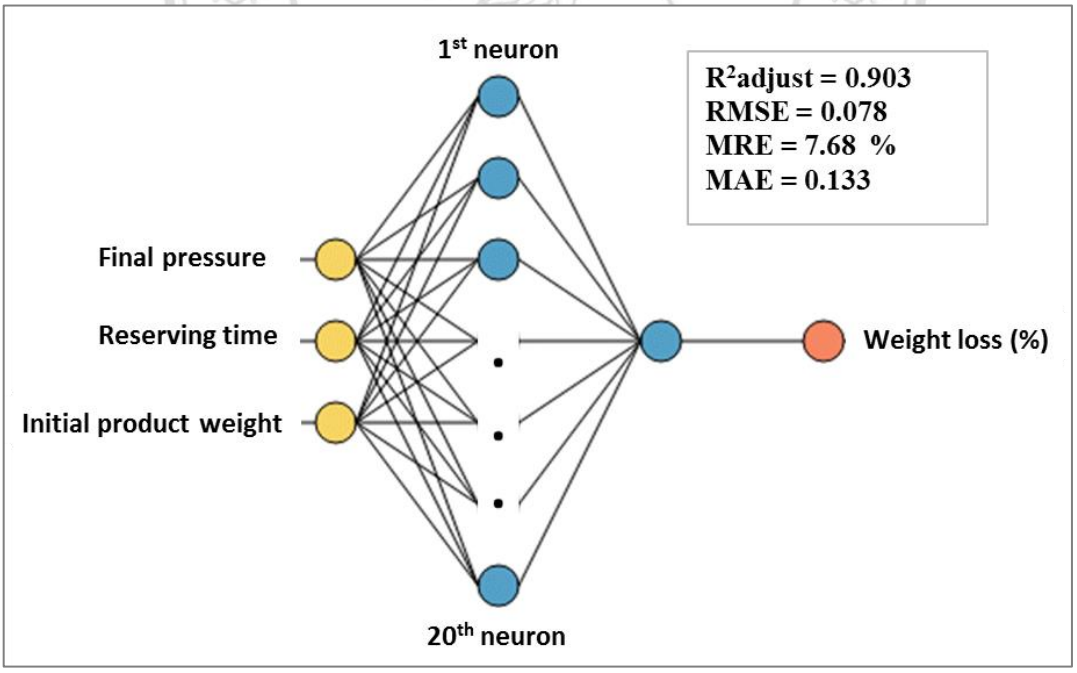

Figure 4.14 The optimum number of hidden layer neurons for weight loss percentage prediction with 3 input parameters

Table 4.3 The optimum neurons in hidden layer for the prediction of final temperature

No. Input	No. neurons	R^2_{adjust}	RMSE	%MRE	MAE
5 inputs	22	0.889	0.084	6.05	0.379
4 inputs	**17**	**0.890**	**0.076**	**10.28**	**0.504**
3 inputs	13	0.753	0.124	16.28	0.872

Table 4.4 The optimum neurons in hidden layer for the prediction of weight loss percentage

No. Input	No. neurons	R^2_{adjust}	RMSE	%MRE	MAE
5 inputs	17	0.793	0.109	9.380	0.173
4 inputs	19	0.767	0.100	9.990	0.201
3 inputs	**20**	**0.903**	**0.078**	**7.680**	**0.133**

4.2.2 Prediction of produce final temperature and weight loss percentage using Multiple Linear Regression (MLR)

The MLR model is a statistical technique for investigating and modeling the relationship between variables and most widely used for analyzing multifactor effects. In almost all applications of regression, the regression equation is only an approximation to the true relationship between the variables and does not imply a cause effect relationship between the variables. Multiple Linear Regression model is a method used to model the linear relationship between a dependent variable and one or more independent variables. The model relating the independent variable to the dependent variable as shown in equation (6)

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \ldots + \beta_n X_n \qquad (6)$$

Where Y = Dependent predicted variables

$X_1, X_2 \ldots X_n$ = Independent variables

$\beta_0, \beta_{1,} \ldots \beta_n$ = Coefficients estimated from the data.

In this study, the independent variables were input parameters obtained from vacuum cooling study in experiment 1 (the same data for ANNs), which consisted of

final pressure (FP), reserving time (RT), Initial temperature (InTp), chamber temperature (ChTp) and Initial weight (InW). The dependent parameters were final temperature and weight loss percentage. After running MLR through the SPSS statistics 17.0 (SPSS Inc., Chicago, USA) calculated using stepwise method. The average for parameter estimates for each output were applied in equation (7) and (8) in order to predicting the final temperature and weight loss percentage from vacuum cooling process.

The ANOVA analysis were carried out to evaluate the statistical significance of the model equation (Table 4.5-4.6). The fit quality was evaluated using R^2_{adjust}, RMSE, MRE and MAE, which compared values predicted by ANNs and the experimented data. MLR models developed to predicted produce final temperature using final pressure (FP), reserving time (RT), initial temperature (InTp), chamber temperature (ChTp) and initial weight (InW) as independent variables in the model as shown in equation (7). The goodness of fit index, namely R^2_{adjust}, RMSE, MRE and MAE from the regression model were 0.503, 0.150, 21.48% and 1.13, respectively (Table A.4). The study has shown that MLR equation cannot be successfully estimate final produce temperature. The best multiple linear regression model for weight loss percentage prediction was displayed in equation (8). Final produce temperature, reserving time, initial temperature, chamber temperature and initial weight were taken into the weight loss percentage prediction. Prediction of weight loss percentage using MLR had higher accuracy than the previous model with the R^2_{adjust}, RMSE, MRE and MAE of 0.481, 0.135, 14.57 and 0.304, respectively (Table A.5)

Final temperature = 0.399 + 0.705FP - 0.698RT - 0.272InTp + 0.119ChTp - 0.360InW + 0.471RT*RT + 1.11InTp*InW (7)

Weight loss (%) = 0.343 + 0.887FP + 0.156RT - 0.958InTp - 0.000014ChTp + 0.121InW - 1.097FP*FP - 1.250ChTp*ChTp - 0.689FP*InW + 0.988RT*ChTp + 2.327InTp*ChTp (8)

Table 4.5 Statistical characteristics and performance measurement of the developed MLR models for final temperature prediction

Final temperature prediction				
Independent parameter	**Estimated coefficients**	**Std. Error**	**T-value**	**α-Level**
(Constant)	0.399	0.183	2.185	0.034
FP	0.705	0.125	5.651	0.000
RT	-0.699	0.245	-2.853	0.007
InTp	-0.272	0.406	-0.671	0.505
ChTp	0.119	0.147	0.809	0.423
InW	-0.360	0.286	-1.259	0.215
RT*RT	0.471	0.257	1.836	0.073
InT*InW	1.110	0.793	1.399	0.169

* Acceptable α-level = 0.1; *T* represents significance of model parameter FP = final pressure, RT = reserving time, InTp = Initial temperature, ChTp =chamber temperature and InW= Initial weight

Table 4.6 Statistical characteristics and performance measurement of the developed MLR models for weight loss percentage prediction

Weight loss percentage prediction				
Independent parameter	**Estimated coefficients**	**Std. Error**	**T-value**	**α-Level**
(Constant)	0.344	0.213	1.617	0.114
FP	0.887	0.566	1.568	0.125
RT	0.155	0.337	0.461	0.647
InTp	-0.958	0.417	-2.295	0.027
ChTp	0.000	0.514	0.000	1.000
InW	0.121	0.190	0.637	0.527
FP*FP	-1.097	0.493	-2.223	0.032
ChTp*ChTp	-1.250	0.620	-2.017	0.050
FP*InW	-0.689	0.412	-1.671	0.102
RT*ChTp	0.988	0.697	1.417	0.164
InTp*ChTp	2.327	0.864	2.694	0.010

* Acceptable α-level = 0.1; *T* represents significance of model parameter FP = final pressure, RT = reserving time, InTp = Initial temperature, ChTp = chamber temperature and InW= Initial weight

4.2.3 Performance comparison of ANNs and MLR

Since the performance between ANNs and MLR models for prediction vacuum cooling outputs, namely final temperature and weight loss percentage were evaluated the accuracy by higher R^2_{adjust} and lower of error (RMSE, MRE and MAE) (63). ANNs models were trained by using multilayers perception with 4 input parameters and 17 neurons in hidden layer demonstrated that the network effectively generates sensitive results and has a sufficient accuracy and reliability rate in modeling final temperature according to high performance than MLR in all goodness of fit index, namely R^2_{adjust} (0.890), RMSE (0.076), MRE (10.28%) and MAE (0.504), respectively (Table 4.7). Moreover, weight loss percentage prediction values obtained by ANNs trained using 3 input parameters and 20 neurons in the hidden had R^2_{adjust}, RMSE, MRE and MAE of

0.903, 0.078, 7.68% and 0.133, respectively (Table 4.8) which indicated higher prediction performance than MLR. As shown from the results, the ANNs model indicated higher prediction performance than MLR model based on evaluation criteria. Moreover, ANNs model had a sufficient accuracy level in the prediction of vacuum cooling outputs. The relationship between the experimental (actual) values and calculated (predicted) values obtained using the ANNs and MLR prediction models were shown in Figure 4.15-4.16 with the difference correlations coefficients (R^2). In the filled plot (Figure 4.15-4.16) presented by ANNs prediction indicated the higher correlation for both final temperature and weight loss percentage (R^2 = 0.8981 and 0.9086) On the other hand, MLR models showed the very low correlation for final temperature and weight loss percentage as shown in Figure 4.17-4.18 with R^2 of 0.5764 and 0.5818, respectively.

Table 4.7 Performance criteria used for predicting final temperature by ANNs and MLR models

Statistic performance	ANN-best fit model	MLR-best fit model
$\mathbf{R^2_{adjust}}$	0.890	0.503
RMSE	0.076	0.150
MRE%	10.28	21.48
MAE	0.504	1.13

Table 4.8 Performance criteria used for predicting weight loss percentage by ANNs and MLR models

Statistic performance	ANN-best fit model	MLR-best fit model
$\mathbf{R^2_{adjust}}$	0.903	0.481
RMSE	0.078	0.135
MRE%	7.68	14.57
MAE	0.133	0.304

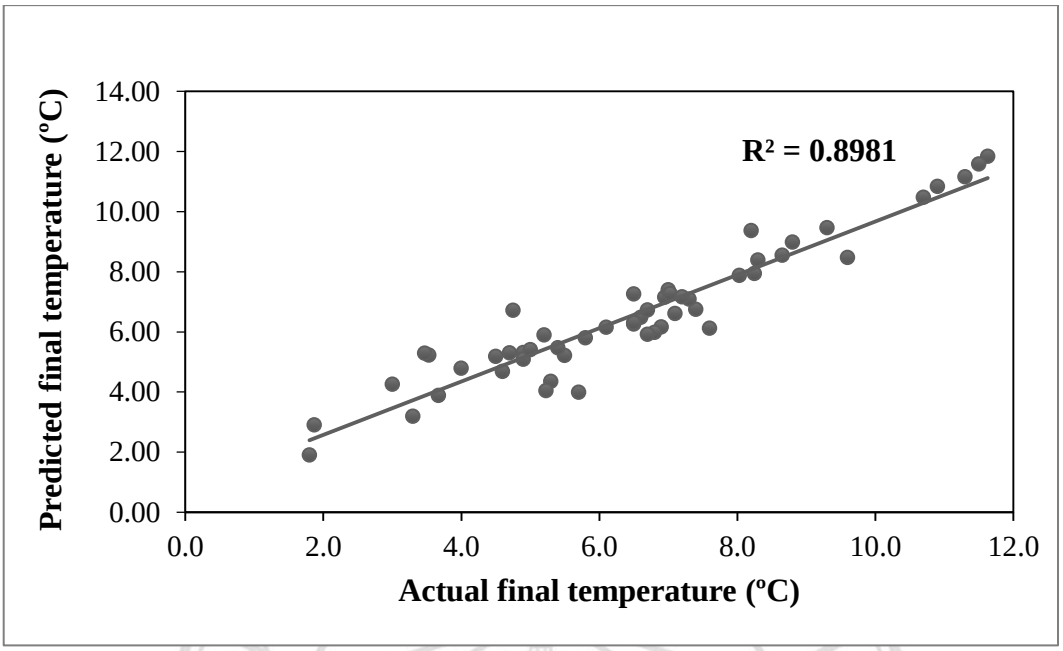

Figure 4.15 Relationship between actual and predicted value of final temperature using the best fit ANNs model

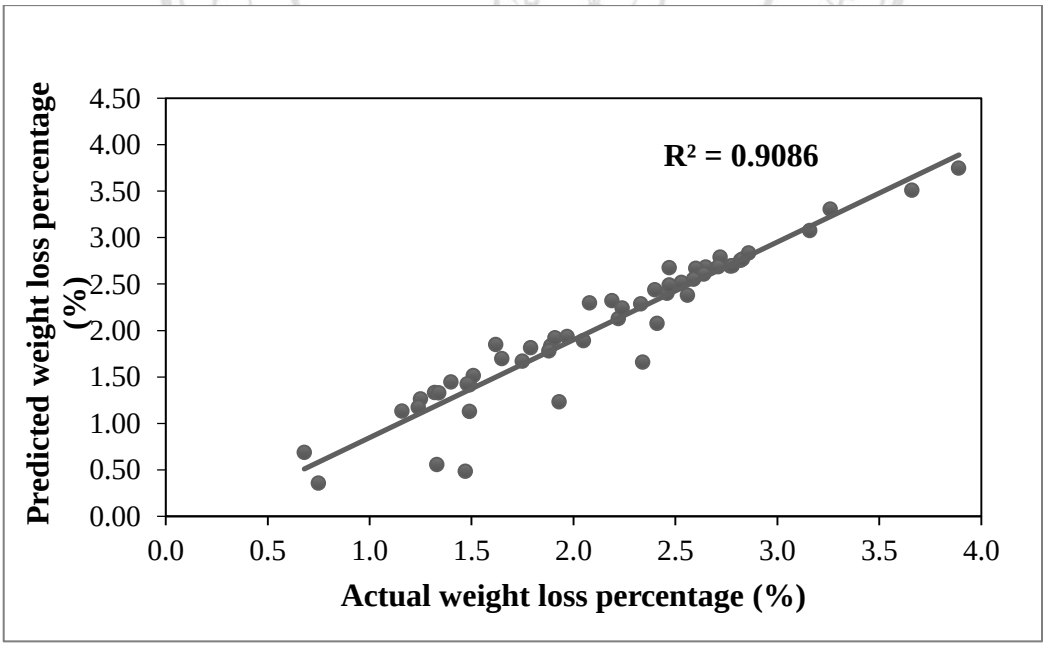

Figure 4.16 Relationship between actual and predicted value of weight loss percentage using the best fit ANNs model

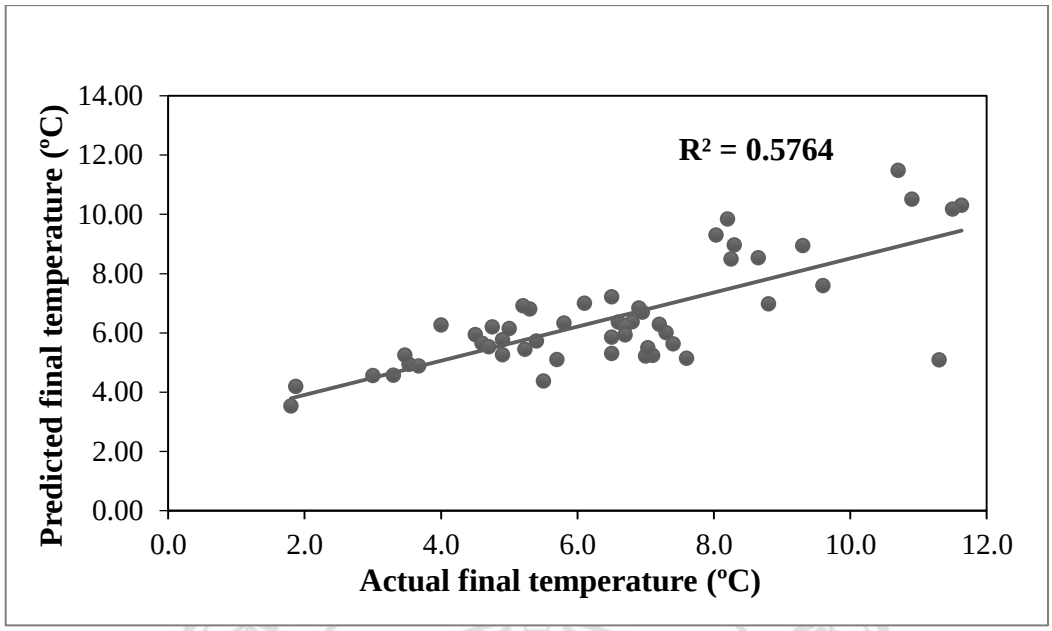

Figure 4.17 Relationship between actual and predicted value of final temperature using the best fit MLR model

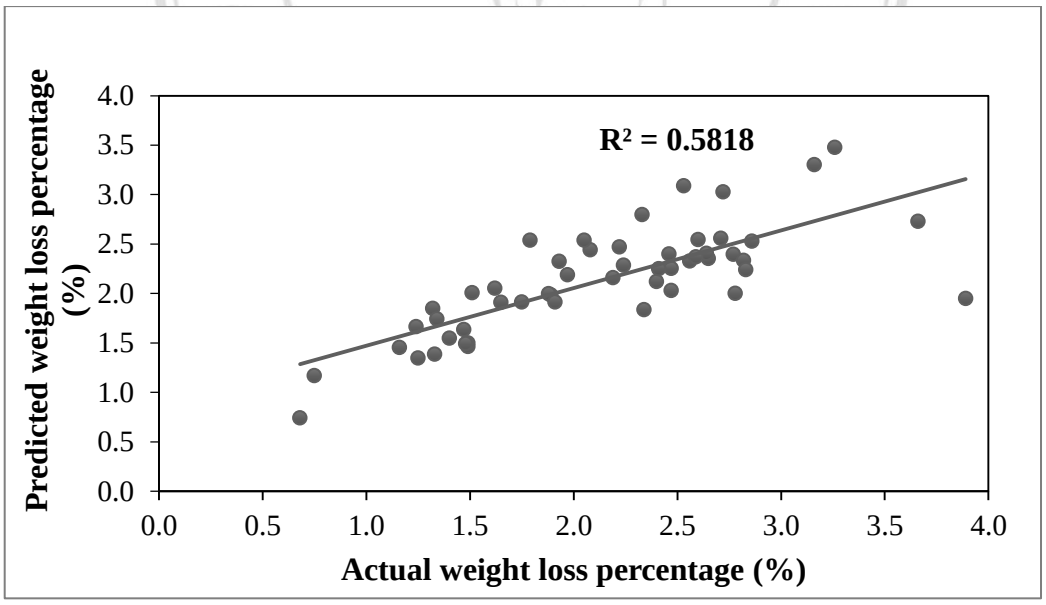

Figure 4.18 Relationship between actual and predicted value of weight loss percentage using the best fit MLR model

Therefore, the best fit model for prediction output of vacuum cooling is the ANNs that correspond to an artificial neural network implemented using a topology (6:20:1) of 6 inputs, 20 neuron in hidden layer and 1 output variable for final temperature prediction and a topology (6:22:1) of 6 inputs, 22 neuron in hidden layer and 1 output variable for weight loss percentage prediction. The developed ANNs model were more accurate to predict final temperature and weight loss percentage for vacuum cooling process of baby cos lettuce based on the initial produce temperature (final pressure, reserving time, initial temperature, chamber temperature and initial weight). However, the ANNs model for the prediction of final temperature and weight loss percentage obtained from this experiment were suggested for vacuum cooling process of baby cos lettuce for industrial scale with qualified produce features include initial temperature 13.4-26.13 °C, initial weight 79-330 kg, final pressure setting of 5-10 mbar with reserving time of 10-30 min, respectively. The models based on multiple linear regressions cannot predict vacuum cooling outputs with similar accuracy as the obtained for the selected neural network. The consistent agreement between the predicted and measured values increases the reliability of the proposed ANNs model for the prediction of vacuum cooling process. It also conclude that a well-trained ANNs model can be useful for vacuum cooling process and applicable without complicated empirical study. Moreover ANNs offered less time and could reduce production costs. Although the comparison performance of ANNs and MLR was found in various applications such as the use of artificial neural networks and multiple linear regression to predict rate of medical waste generation, predicting compression strength of heat treated woods (64), interpretation of concrete dam behavior (65), ozone concentrations prediction (66), prediction of the cetane number of biodiesel (67). However, results of mentioned researchs indicated the same trend and agreed well with this study which confirmed the high performance of ANNs over MLR model.

4.3 Experiment 3: Effect of vacuum cooling on qualities of baby cos lettuce during storage compare with the forced-air cooling and room cooling

4.3.1 Precooling performance evaluation

This experiments were conducted in order to compare the effect of precooling method (vacuum cooling, forced-air cooling and room cooling) on qualities of baby cos lettuce. All type of precooling methods were carried out with the industrial scale including vacuum cooler, forced-air cooler and cold room at the Royal Project Foundation, Thailand. There are different cooling parameters among the precooling methods which precooled baby cos lettuce from the initial temperature around 21-25 ºC to the final temperature of 4±1 ºC. Vacuum cooling showed the high performance with shorter cycle time 36 min and faster cooling rate of 0.458 ºC/min followed by forced-air cooling which demonstrated the medium cycle time of 120 min and medium cooling rate of 0.177 ºC/min. On the other hand, room cooling indicated the longer cycle time of 525 minutes with very slow cooling rate of 0.038 ºC/min (Table 4.9). The results agreed with precooling of artichokes using three different pre-cooling methods such as air blast, hydro-precooling and vacuum. The vacuum precooling method has the shortest precooling time of 35 minutes followed by hydro precooling with 58 minutes cooling time and air precooling with 135 minutes (68).

Generally, precooling methods for fresh product are room cooling, where the produces are cooled by placing them into cold room or still air refrigerant. Forced-air cooling, where the produces are cooled by forcing refrigerator air through them with optimum air flow rate. Vacuum cooling, where the products are cooled by vaporizing some of the water content of the products under low pressure conditions and suitable for any produce which have a large surface area for mass transfer (water evaporation) and free water containing. The cooling effect comes from water boiling from the samples, and therefore evaporation and cooling of the sample started from the surface. On the other hand, room cooling and forced-air cooling product were cooled by utilized heat transfer through the medium or convection heat transfer. Although the results showed that the vacuum cooling was a rapid and efficient cooling method when compared with forced-air cooling and room cooling following by 3.25, 2.75 and 1.87%, respectively. There was some weight loss occurred during vacuum cooling since cooling effect directly comes from water evaporation (boiling) from produce. This could explain for

vacuum cooling the amount of heat removed from the produce is proportion to the mass of water evaporated and the heat of vaporization of water at the average temperature, therefore, vacuum cooled produce lose 1 % of moisture content for each 6 ºC dropped in their temperature (69).

The energy consumption of the difference precooling methods were compared using the processing cost per amount of produce. The results indicated that the energy consumption was related to the cooling time which vacuum cooling showed the lowest energy consumption of 3.40 kWh. Corresponding to the lowest cooling time. On the other hand, forced-air cooling and room cooling demonstrated the higher energy consumption of 33.20 and 44.28 kWh, respectively, due to the longer cooling time and the load of equipment such as compressor, condenser and the blower. Our results agreed well with the other research which found that vacuum coolers are the most efficient when compared with hydrocooler and forced-air cooler (70). The total cooling time of vacuum cooling was depending on some important factors such as the shape of the product, porosity, pore size and the pore distribution within the samples, and the availability of free water in the pores, as well as the parameters setting of vacuum cooling. When compared with room cooling and forced-air cooling, vacuum cooling had cooling time faster than forced-air cooling 3.33 times and 14.58 times than room cooling (Figure 4.19). The same results were found when using the vacuum cooling with broccoli which indicated the greatly shortened cooling time of vacuum cooling than ice-water cooling and cold room cooling (71) as well as vacuum cooling of cauliflower heads which indicated the lowest cooling time and the lowest energy consumption, followed by high and low flow hydro and forced air precooling methods (31).

Table 4.9 Cooling parameters of difference precooling methods

Cooling parameters	Vacuum cooling	Forced-air cooling	Room cooling
Initial temperature (°C)	22.47 ± 0.47	24.29 ± 0.38	21.0 ± 0.20
Final temperature (°C)	5.77 ± 0.25	5.73 ± 0.44	5.39 ± 0.54
Cooling time (min)	36	120	525
Weight loss (%)	3.25	2.75	1.87
Cooling rate (°C/min)	0.458	0.177	0.038
Energy consumption (kWh)	3.40	33.20	44.28

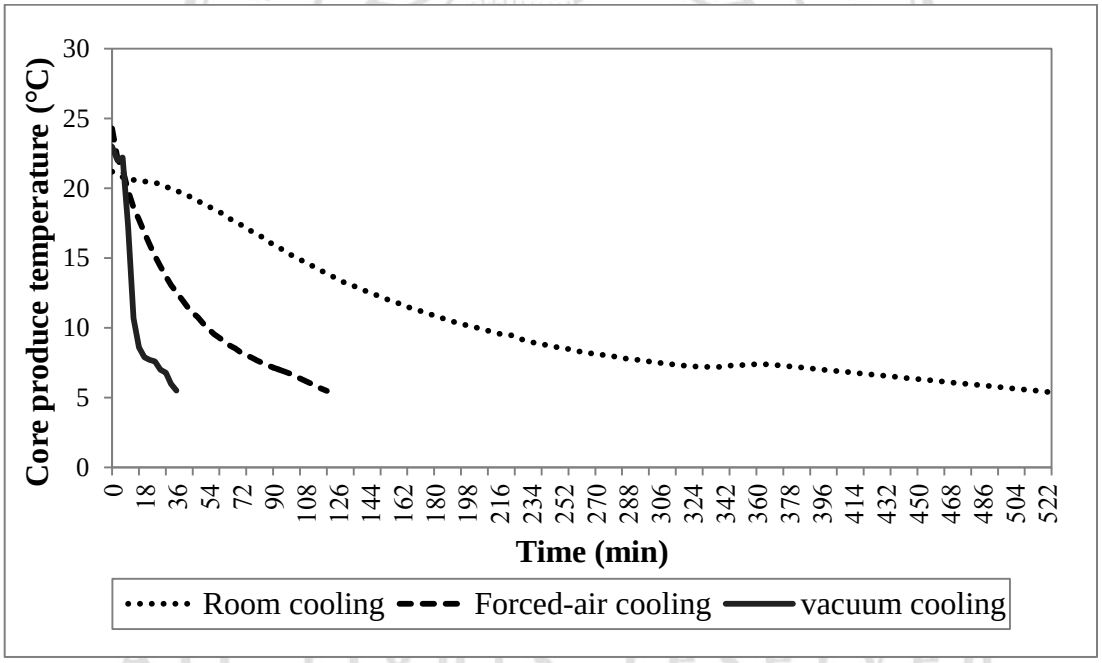

Figure 4.19 Cooling rate of difference precooling methods of baby cos lettuces

4.3.2 Physical qualities of precooled baby cos lettuce during storage

4.3.2.1 Weight loss

The loss of weight increased continuously during storage, being higher weight loss percentage on the first day of storage, it was found significant differences observed in control and precooling treatment ($p \leq 0.05$) which control exhibited the lower weight loss percentage than precooling treatment. However, after 9 days storage significant differences were obtained depending on the treatment. Thus, baby cos lettuces precooled with forced-air cooling and room cooling exhibited significantly higher weight loss percentage than control and vacuum cooling from throughout storage ($p \leq 0.05$) due to the samples were exposed to high air flow rate of 0.8-2.0 m/s for a long time (forced-air cooling for 120 min and room cooling for 525 min) (Table 4.10). Rennie *et al*. (2001) suggested that vacuum cooled lettuce with difference pressure reduction rate stored at 1°C and 85% RH indicated that the weight loss with respect to time was nearly linear with mass loss percentage after 9 days of storage ranging from 1.6 to 1.7 %. In addition, the final mass loss percentage after 16 days ranged from 2.7 and 3.2 % . On the other hand, the rate of pressure reduction had no effect on the subsequent storage mass loss of the lettuce (72). Leafy vegetables during postharvest storage lose the fresh weight mainly due to transpiration as they generally have a large surface to volume ratio, which makes them vulnerable to rapid water loss after harvest (73). Transpiration regulation through the stomata was well understood in the case of leaves. Forced-air cooling had been demonstrated to stimulated stomata opening and moreover, the numbers of open stomata have been well correlated with the fresh weight loss of baby cos lettuces. Furthermore, some environment factors could effect on water loss following by relative humidity, the temperature surrounding product and air velocity (74).

4.3.2.2 Texture

Baby cos lettuce contains two different types of tissue (vascular and photosynthetic) which were not always easy to differentiate. Normally the tissue had an irregular distribution. Therefore, instrumental measurement of baby cos lettuce texture is difficult to carry out, due mainly to the high variability of the produce (75). Different behaviors of the load–displacement profile using the Kramer cell were observed

depending on the crispness of the samples. The use of a coefficient based on the maximum load and the minimum load immediately after the breaking point, which was called crispiness coefficient (CC) which was a useful tool to distinguish between treatments, which could not be distinguished by means of the ratio maximum load/weight (76). The results indicated not significant differences observed at day 0. The similar trend was found in the other research indicated that Romaine and Iceberg lettuce treated with processing conditions (cutting, treatment, and modified atmosphere packaging) were compared texture against an untreated raw sample (77). However, at the first day of storage significantly higher values ($p \leq 0.05$) of CC were observed in precooled sample treatment (Table A.6). At day 9 of storage all precooling treatment exhibited higher CC values than control sample ($p \leq 0.05$) but there are no significant difference between precooling treatment from day 9 until the end of storage (Table 4.10). The precooled samples showed higher CC values than control, which was interpreted as a higher crispness (Table 4.10). The results agree with He *et al.* (2004) conclude that after 2 weeks of cold storage at 1 °C and 85% RH the firmness of head lettuce reduced with storage time and vacuum cooling assisted in maintaining the texture of lettuce which indicate higher peak force (N) than lettuce sample without vacuum precooling (30). The decrease in crispy characteristic textural properties of lettuce related to the loss of turgor of the cells after treatment due to dehydration (78). However, there are not significant differences among the precooling method may be due to more difficult instrumental measurements to detected the different tissue types and heterogeneity of the samples (79). Moreover, Our observation was similar to the instrumental measured firmness of packaged, shredded iceberg lettuce stored up to 14 days at 3 °C (80) which found not significant change of firmness at the end of storage.

4.3.2.3 Overall visual quality

Fresh appearance is the main attribute that consumers use to evaluate the quality of vegetables and fruits, since people “buy with their eyes”. Appearance, browning and texture are key aspects used in sensory analysis to evaluate the general quality of a product. For baby cos lettuce browning and lack of crispiness are critical factors in perceived loss of quality. Sensory analysis by five panelists was used to assess the quality of baby cos lettuce during cold storage for 16 days (Table 4.10). The results

showed that there is no significant difference found on the first day of storage. However, after 9 days of storage, the overall quality of vacuum cooled, room cooled and forced-air cooled samples were 6.80, 6.40 and 6.40, respectively which were significantly higher ($p \leq 0.05$) than the control sample (5.40). The cut off of trained panelists acceptability was 6.0, therefor the end of shelf life of control sample was after 9 days of storage. After 13 days of storage, the overall visual quality score of forced-air cooling and room cooling samples demonstrated the score of visual quality of forced-air cooling (5.20) and room cooling (5.20), which significant lower ($p \leq 0.05$) than vacuum cooling samples (6.25). The cut off score (6.00) indicated the limit of trained panelists acceptability. On the other hand, higher fresh appearance values were observed in samples precooled with vacuum cooling after day 15 of storage (Figure 4.20). Therefore, the shelf life of baby cos lettuce base on consumer acceptability score for control was 9 days, forced-air cooling and room cooling were 13 days as well as vacuum cooling was 15 days. Roger (2012) supported that vacuum cooling improved shelf life compared to forced air cooling the resulted proved that vacuum cooling of cos lettuce within half an hour of harvest had shelf life during storage at 5 ºC for 13 days, whereas cos lettuce samples that was forced-air cooled achieved 11 days of storage under the same condition (81). The results was agree well with vacuum precooled cauliflower heads stored under room conditions (22±1 °C and 55-60% RH) for 10 days were rated as "*saleable*" (score 5), whereas the precooled heads using the low and high flow hydro-cooling method were rated as "unsaleable" (scores 4 and 3, respectively) and the overall sensory quality score of the forced-air precooled and non-precooled cauliflower heads were rated "*unavailable*" with (scores 2 and 1, respectively) (32).

4.3.2.4 Color Change

The color of baby cos lettuce leaves and cut surface were measured separately during cold storage. The leaves of precooled samples showed the same trend as the control sample (all color parameters) during the entire storage period. Precooling method have an effect on the hue angle values of the leaves (Figure 4.21-4.23). The hue angle describes the quality of the color of the produces. All precooled treatments showed higher values of hue angle than control samples which means precooled samples have more green color than control. Color determining by lightness or degree

which an object reflects light, and chroma or saturation, which is the intensity of color or difference from gray of the same lightness (82). The chroma values of leaves and cut surface remained constant in all treatment for the entire storage period and forced-air cooling treatment exhibited the lower of chroma due to higher loss of water affect to wilting and poor appearances (Figure 4.24-4.26). Salgado *et al.* (2014) found that different chemical and ultrasound treatments had little effect on color change of lettuce during storage at 4 ºC for14 days (77). The color readings all have relatively large standard errors, which attribute to the heterogamous composition of different tissues in lettuce samples (79). The cut surface color of sample showed higher losses in color than the leaves. The color changes of cut surfaces was mostly due to decrease in hue angle, which expressed in increasing of red color and decreasing L*value caused more intense of darkening color. This results might be explained by oxidation of phenolic compounds. Loss of color quality is mainly from enzymatic browning reaction due to wounding, which caused by the accumulation and oxidation of phenols by polyphenoloxidases and peroxidases to *O*-quinones that polymerize readily into dark pigments (54). The vascular tissues of iceberg lettuce developed brown discoloration at cut edges more quickly than photosynthetic tissue when compared h° and a* values with visual ratings of browning separately on vascular and photosynthetic tissue (79).

Table 4.10 Weight loss, texture and overall visual quality of baby cos lettuce during storage at 4 °C with 85 % RH

Storage period	***Treatment***	***Weight loss (%)***	***Texture (KN/Kg) (Crispness coefficient)***	***Overall visual quality (score)***
0 day	Control	-	0.894 ± 0.033^{a}	8.40 ± 0.55^{a}
	Forced-air	-	0.905 ± 0.013^{a}	8.40 ± 0.89^{a}
	Vacuum	-	0.899 ± 0.018^{a}	8.40 ± 0.55^{a}
	Room	-	0.902 ± 0.006^{a}	8.40 ± 0.55^{a}
9 days	Control	0.67 ± 0.17^{c}	0.862 ± 0.021^{b}	5.40 ± 0.55^{b}
	Forced-air	1.27 ± 0.21^{b}	0.889 ± 0.016^{a}	6.40 ± 0.55^{a}
	Vacuum	0.72 ± 0.80^{a}	0.894 ± 0.018^{a}	6.80 ± 0.45^{a}
	Room	1.59 ± 0.13^{a}	0.905 ± 0.017^{a}	6.60 ± 0.55^{a}
13 days	Forced-air	1.79 ± 0.21^{b}	0.875 ± 0.026^{a}	5.20 ± 0.45^{b}
	Vacuum	1.16 ± 0.07^{c}	0.881 ± 0.011^{a}	6.25 ± 0.50^{a}
	Room	2.15 ± 0.22^{a}	0.863 ± 0.017^{a}	5.20 ± 0.45^{b}
15 days	Vacuum	1.42 ± 0.03	0.885 ± 0.015	6.00 ± 1.00
16 days	Vacuum	1.69 ± 0.09	0.883 ± 0.015	5.60 ± 0.50

Values designated by the same letter are not significantly different ($p > 0.05$). Lower case letter are used for comparisons during storage.

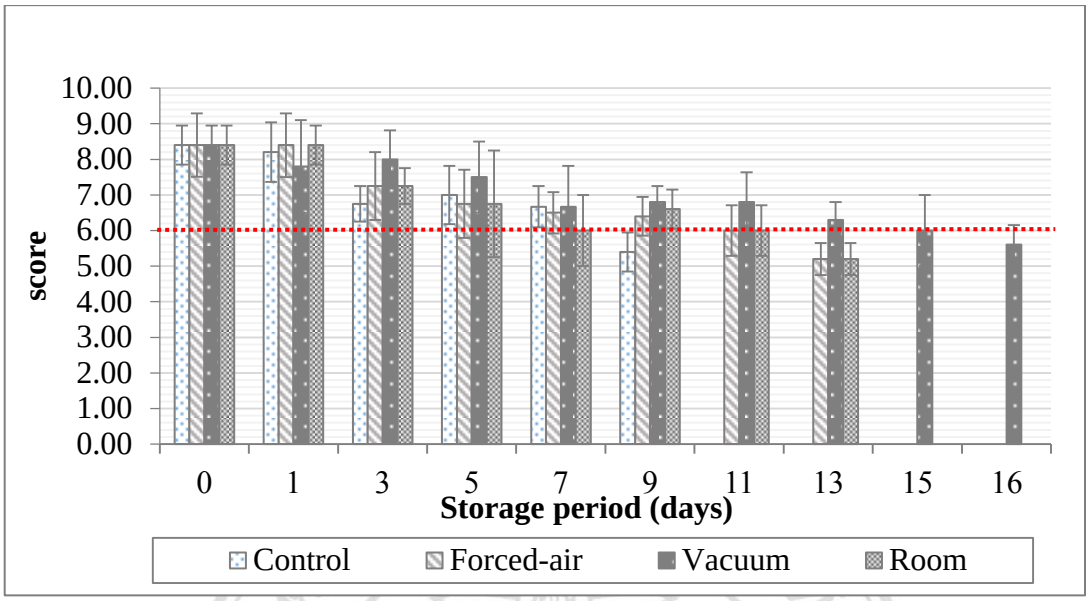

Figure 4.20 Changes in visual quality of baby cos lettuce during storage at 4 °C with 85 % RH (Dash line represents the limit of marketability)

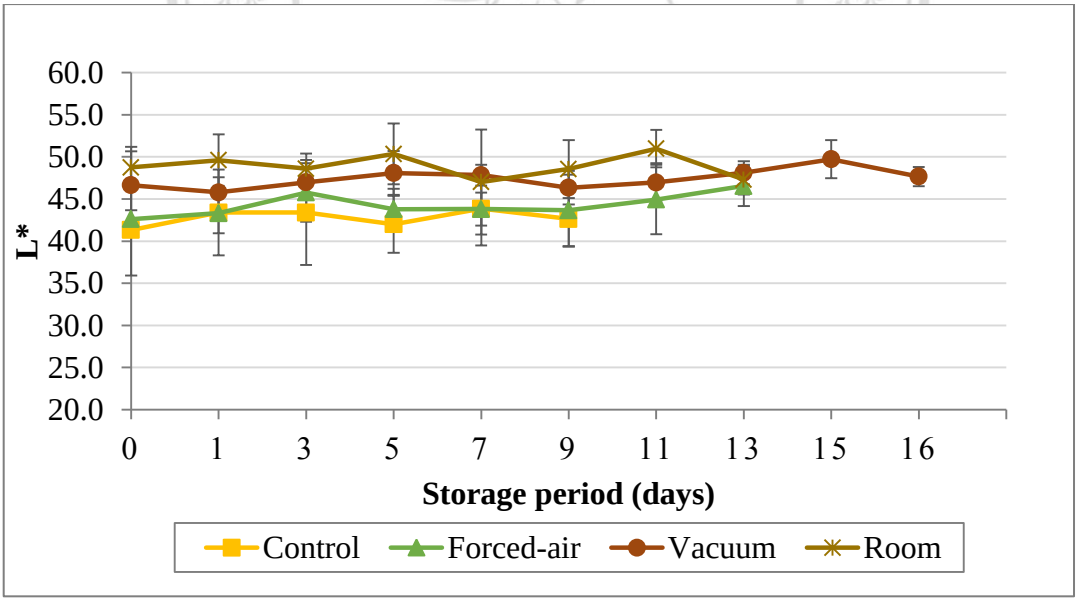

Figure 4.21 L* value of baby cos lettuce leave during storage at 4 °C with 85 % RH

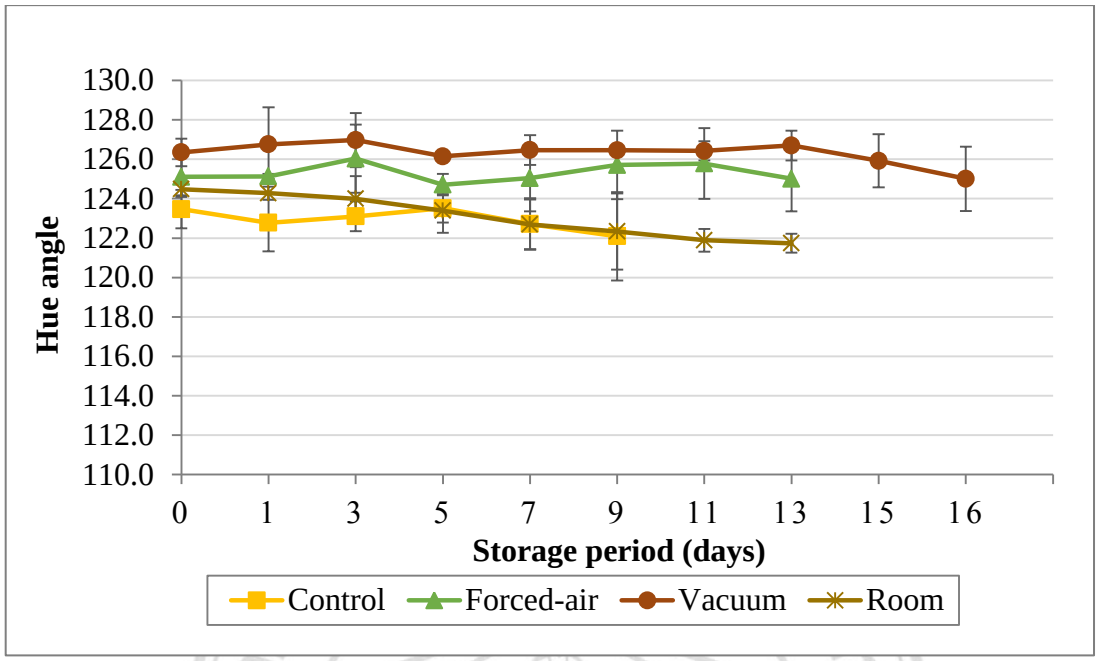

Figure 4.22 Hue angle of baby cos lettuce leave during storage at 4 °C with 85 % RH

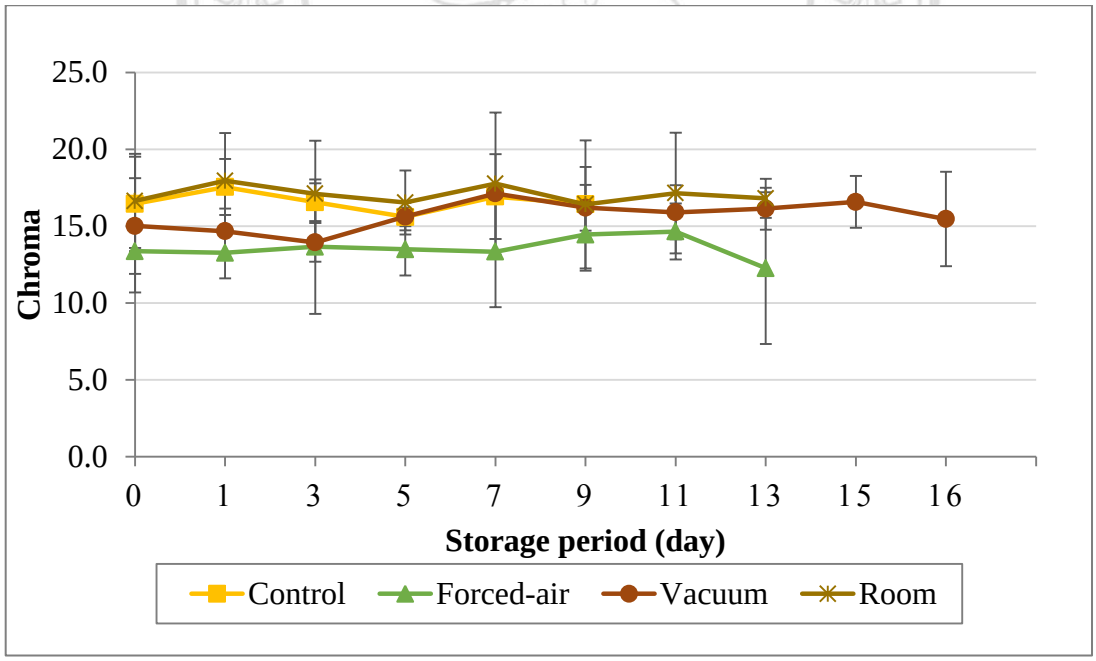

Figure 4.23 Chroma of baby cos lettuce leave during storage at 4 °C with 85 % RH

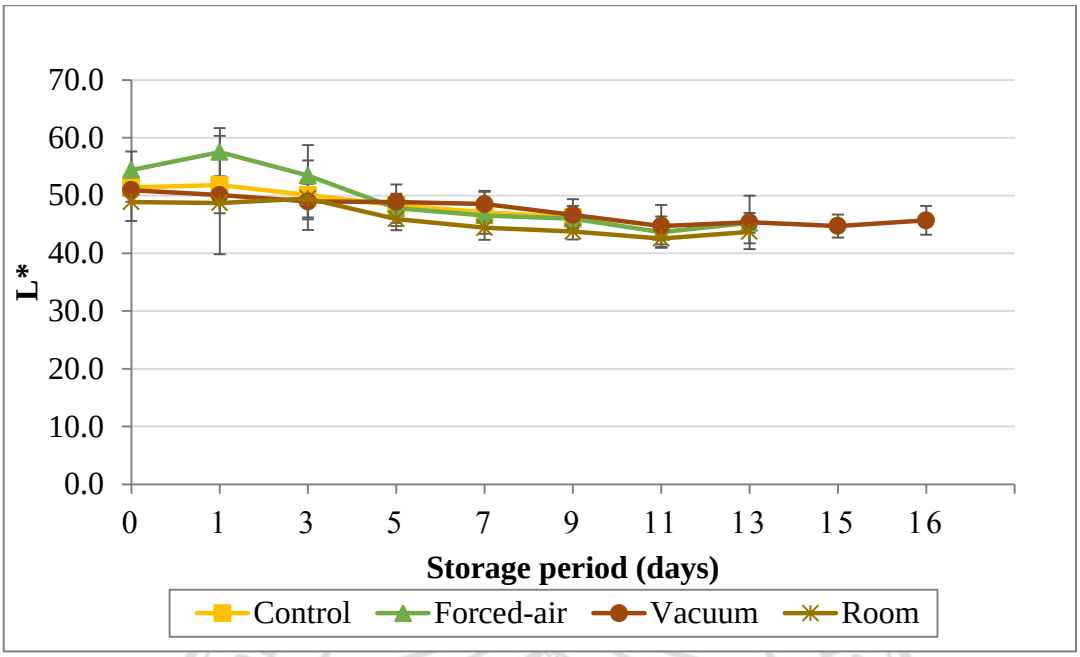

Figure 4.24 L* value of baby cos lettuce cut surface during storage at 4 °C with 85 % RH

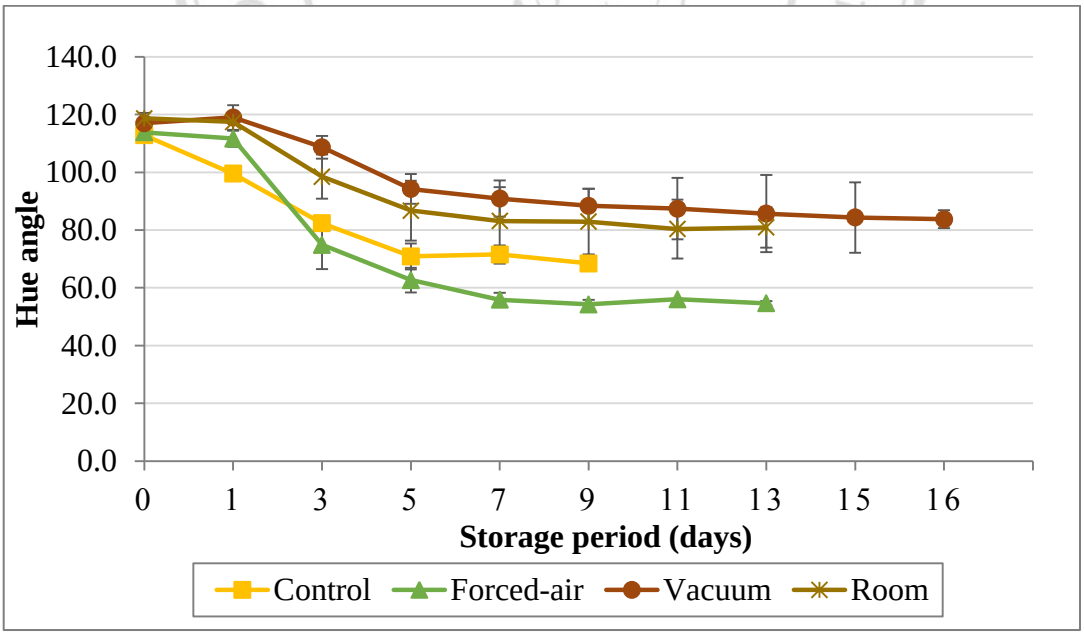

Figure 4.25 Hue angle of baby cos lettuce cut surface during storage at 4 °C with 85% RH

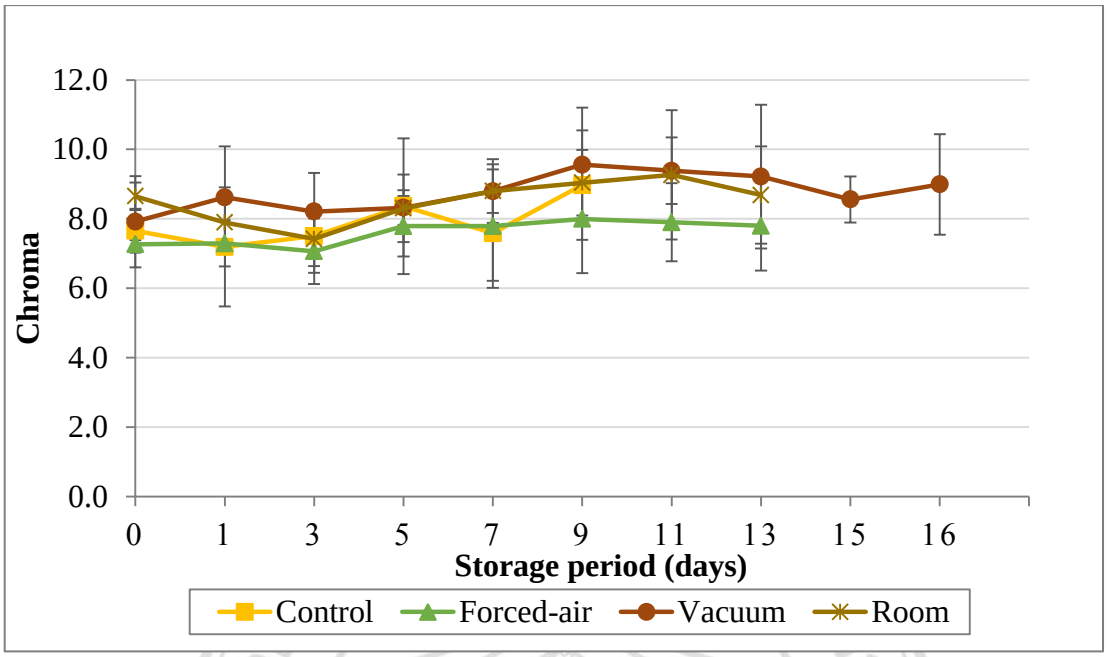

Figure 4.26 Chroma of baby cos lettuce cut surface during storage at 4 °C with 85 % RH

4.3.2.5 TEM measurement for ultrastructure

The baby cos lettuce samples were scanned using transmission electron microscopy (TEM) images analysis. Treatments were collected immediately after precooling treatment (vacuum cooling, forced-air cooling and room cooling) and after stored in cold storage for 15 days which compared to control group. Right after precooling method the ultrastructure of all treatments demonstrated the integrity of cells except the sample from control treatment (Figure 4.27-4.30) The cell from precooled sample exhibited intact cells and well defined organelles such as chloroplast with stacked thylakoids and vacuole (83). The fresh leave tissue showed chloroplast peripherally with abundant grana and intergrana lamella and frequently aligned in the limit of cytoplasm, near the internal face of the cell membrane (84) and vacuole are usually empty looking spaces compared with the surrounding cytoplasm and occupy 90% or more of the volume of cell in mature tissues (85). However, baby cos lettuce precooled with forced-air cooling, vacuum cooling and room cooling exhibited plasmolysis due to water loss after precooling treatment (Figure 4.28, 4.29 and 4.30). The predicament is called “plasmolysis” showed the contraction of the protoplast of plant cell and leaving the large spaces between the plasma membrane and cell wall (86). Plasmolysis appearance of cells suggested the water moved out of cell as a results of

water loss from the cell. After 13 days of storage, the senescence phenomenal was observed in control, forced-air cooling and room cooling treatment. The senescence of samples were detected by TEM illustrated as chloroplast degradation. The fresh baby cos lettuce cell exhibited the membrane envelope surround the chloroplast stomata within stacked grana thylakoids (87) (Figure 4.32). After 13 days of cold storage, control and room cooling samples showed the degradation of vacuole and disappearance of organelle in cell (Figure 4.31 and 4.37). Moreover, the degradation of chloroplast was found in control, forced-air cooing and room cooling treatments indicated the losing of thylakoid stacks and exhibited the electron dense plastoglobuli are visible as black spheres (84, 88) (Figure 4.34-4.37). The previous research (89) suggested that young chloroplasts contained free of osmiophilic plastoglobuli with small size or show up in a lower number with rather large size. In senescing chloroplasts and in their final form, gerontoplasts, thylakoids and chlorophylls are successively broken down with formation of large plastoglobuli. Besides the senescence of cell, the TEM captured the large crystal (black square shape) was found in control, forced-air cooling and room cooling (Figure 4.33, 4.34, 4.36 and 4.37) which implied the activity of catalase enzyme occurred throughout the peroxisome where the peroxisome membrane approach the neighboring chloroplasts. There might be high catalase activity in regions of close contact where transport of molecules between chloroplast and peroxisome (85). Catalase is a common enzyme present mainly in the peroxisome of cells function as an antioxidant when tissue suffer with stress, they can minimize free radical and protect organism.

On the other hand, the ultrastructure of vacuum cooling treatment exposed the integrity of chloroplast and the grana lamella of chloroplast were clearly seen. Moreover, cell wall was intact after 13 days of storage (Figure 4.38 and 4.39). It could be explained the consumer acceptability score of baby cos lettuce that vacuum cooling showed the higher score than control and forced-air cooling at the end of storage. The TEM results related to the previous study (30) which determined the effect of pressure reduction rate on vacuum cooling qualities of head lettuce. The research found the variation of ultrastructure including plasmolysis, irregular membrane structure and discontinuity of plasmalemma and tonoplast especially the lowest pressure reduction rate treatment which exposed to the low pressure ambient for a long time (60 min).

When storage for 2 weeks the lettuce cooled at the moderate pressure reduction rate showed intact organelles, those vacuum cooled at the lowest and highest pressure reduction rates had damaged organells.

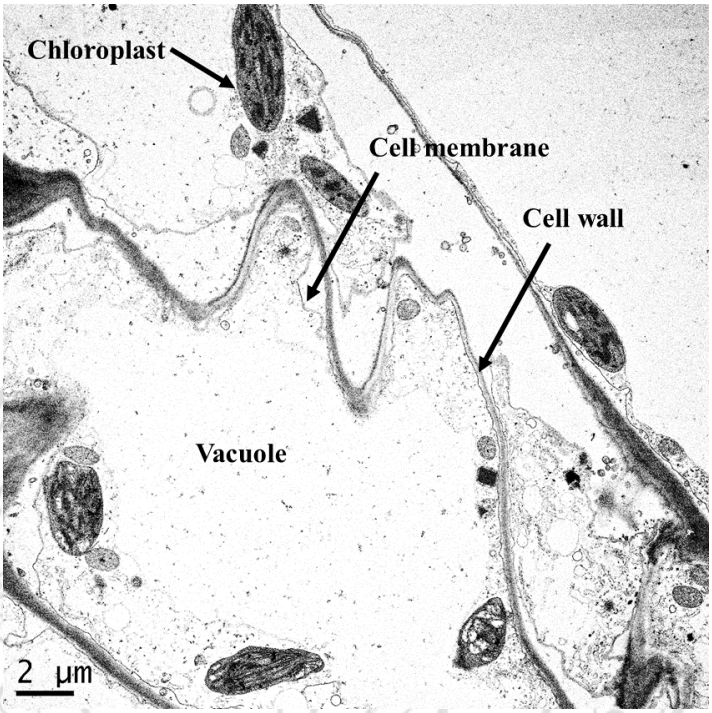

Figure 4.27 Transmission electron microscope images of baby cos lettuce cell in control treatment at day 0 of storage

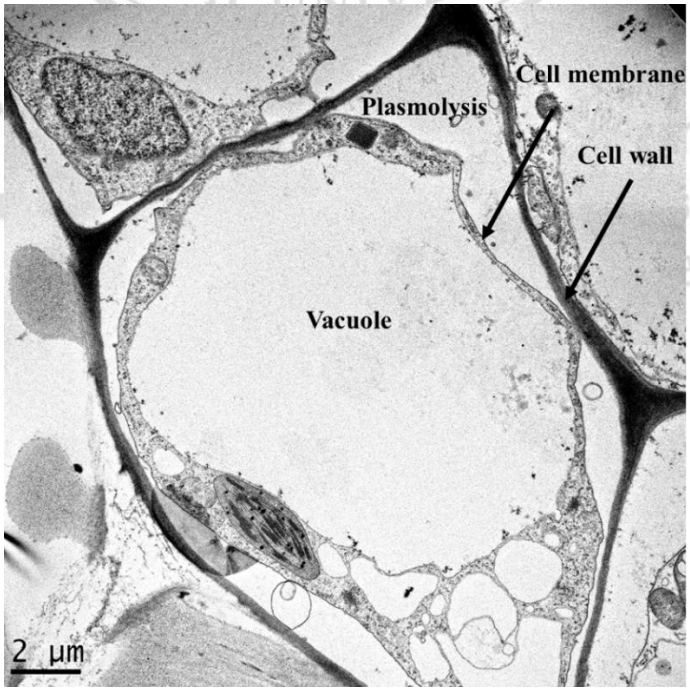

Figure 4.28 Transmission electron microscope images of baby cos lettuce cell after forced-air cooling

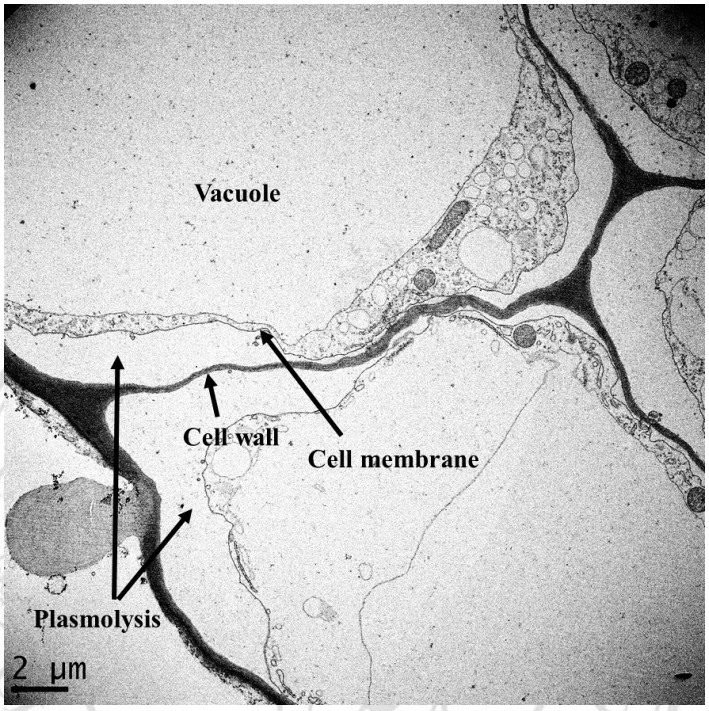

Figure 4.29 Transmission electron microscope images of baby cos lettuce cell after vacuum cooling treatment

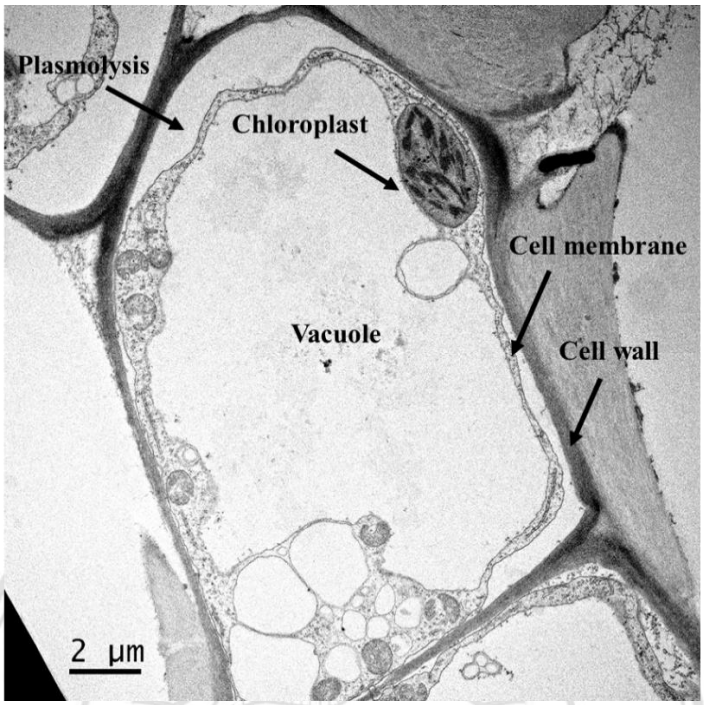

Figure 4.30 Transmission electron microscope images of baby cos lettucecell after oom cooling

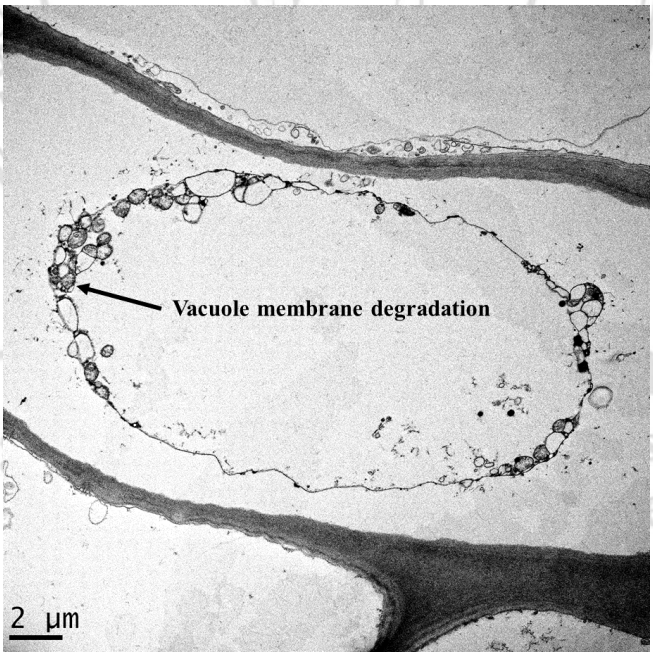

Figure 4.31 Transmission electron microscope images of baby cos lettuce cell in control treatment after 13 days of storage

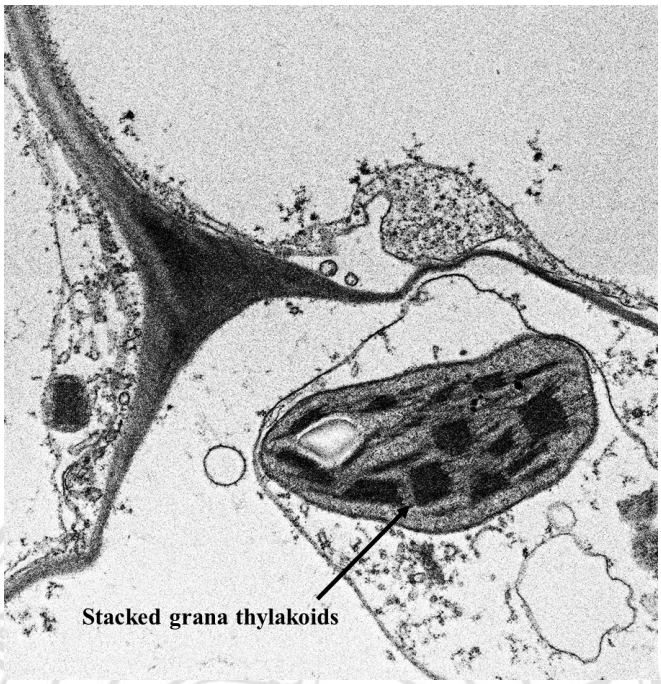

Figure 4.32 Transmission electron microscope images with higher magnification view of chloroplast in baby cos lettuce cell at day 0 of storage

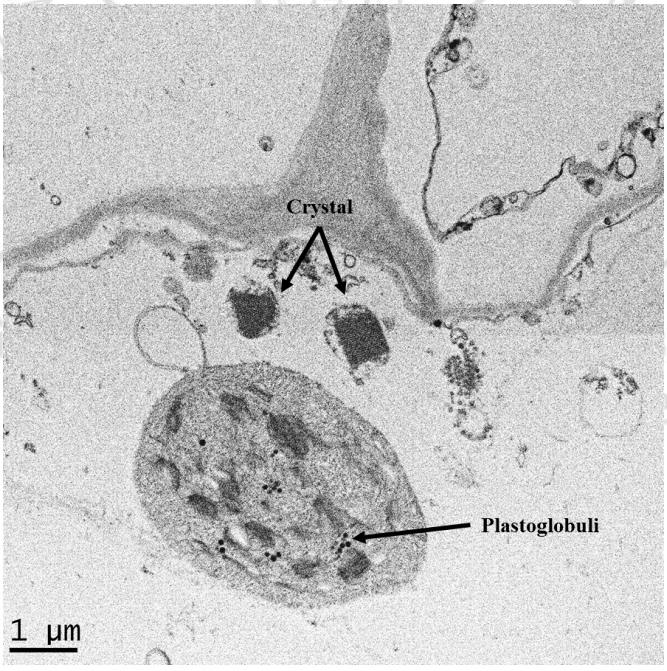

Figure 4.33 Transmission electron microscope images with higher magnification view of precooled baby cos lettuce cell with

forced-air cooling after 13 days of storage (Electron dense plastoglobuli are visible as black spheres)

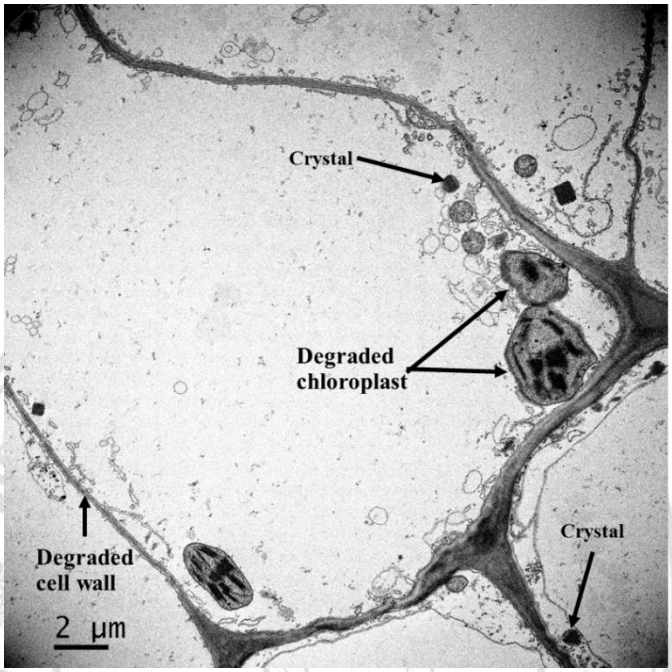

Figure 4.34 Transmission electron microscope images of baby cos lettuce cell in control treatment after 13 days of storage

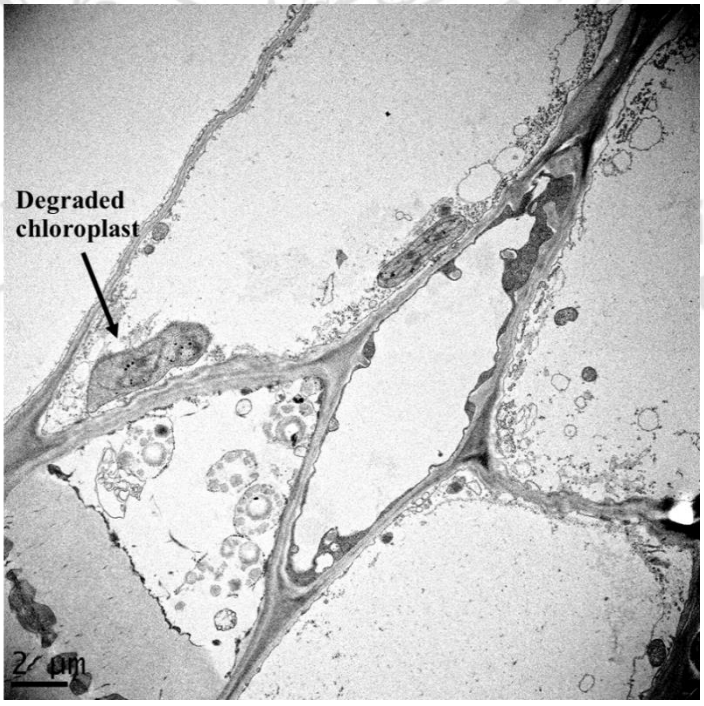

Figure 4.35 Transmission electron microscope images of precooled baby

cos lettuce cell with forced-air cooling after 13 days of storage

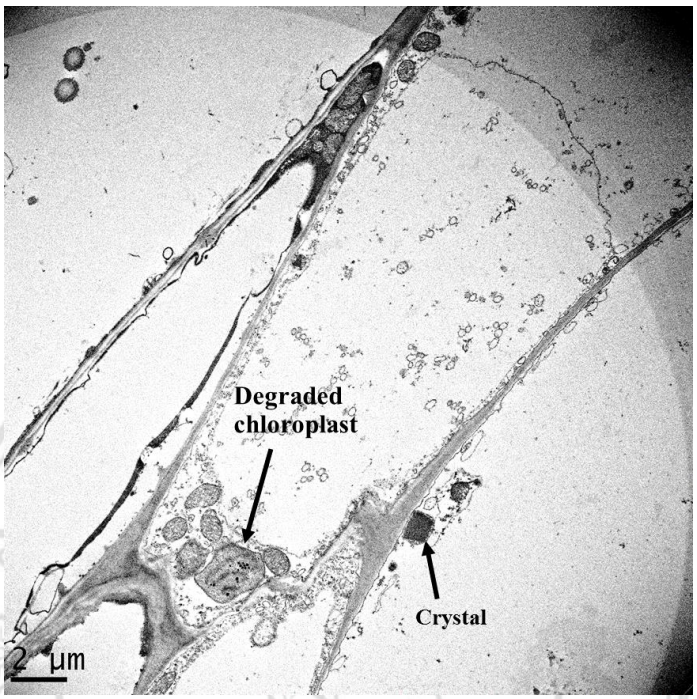

Figure 4.36 Transmission electron microscope images of precooled baby cos lettuce cell with forced-air cooling after 13 days of storage

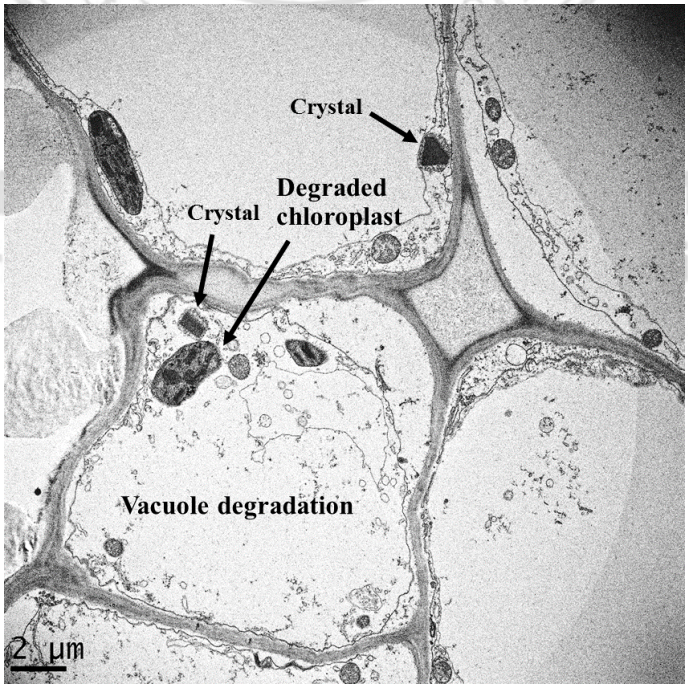

Figure 4.37 Transmission electron microscope images of precooled baby cos lettuce cell with room cooling after 13 days of storage

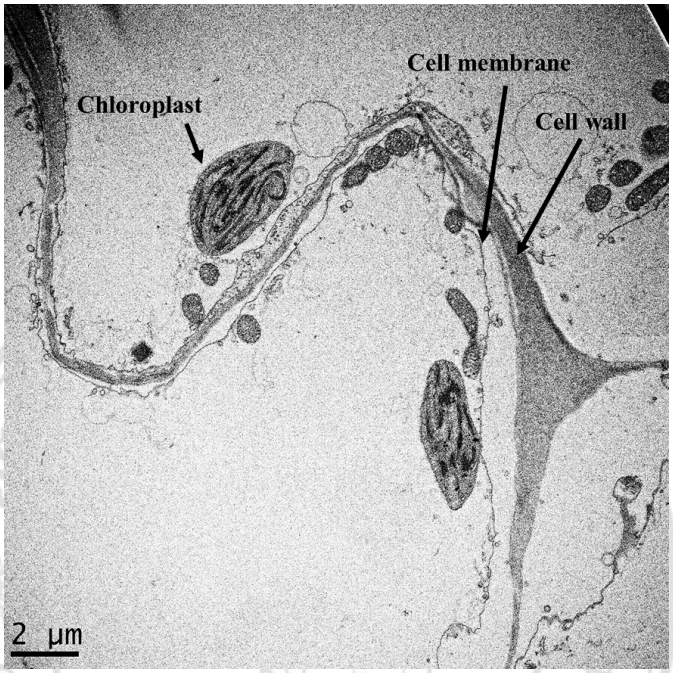

Figure 4.38 Transmission electron microscope images of precooled baby cos lettuce cell with vacuum cooling after 13 days of storage

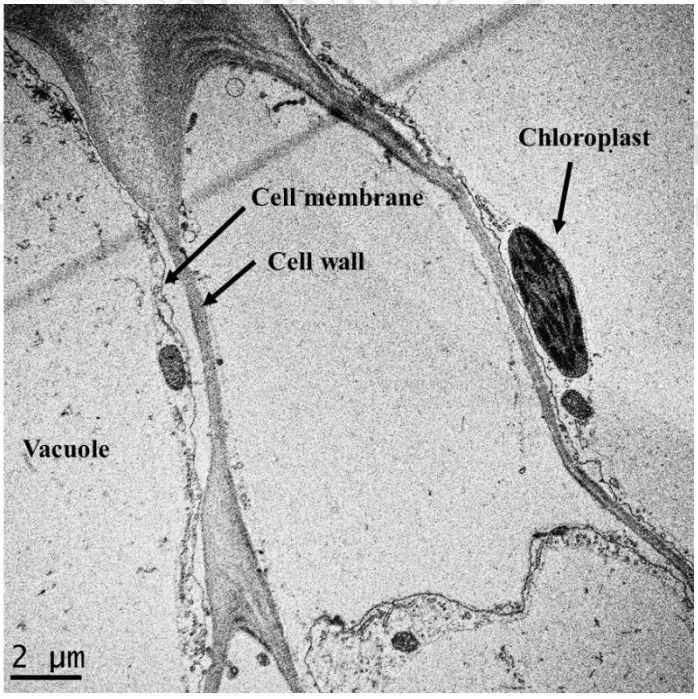

Figure 4.39 Transmission electron microscope images of precooled baby cos lettuce with vacuum cooling after 13 days of storage

4.3.3 Chemical qualities of precooled baby cos lettuce during storage

4.3.3.1 Chlorophyll content

On the first day of storage, the chlorophyll content of baby cos lettuce ranged from 46.46 to 49.37 µg/g FW (Table 4.11). During cold storage at 4 ºC with 85%RH, the chlorophyll content declined very quickly in all treatments. After day 9 of storage, vacuum cooling samples maintained the highest chlorophyll content (45.41 µg/g FW) which was significantly different ($p \leq 0.05$) compared to forced-air cooling (42.75 µg/g FW), room cooling (35.95 µg/g FW) and control (33.37 µg/g FW). At the end of storage (day 13), the statistical results indicated significant difference ($p \leq 0.05$) that vacuum cooling (32.53 µg/g FW) obtained higher chlorophyll content than forced-air cooling (29.90 µg/g FW) and room cooling (28.56 µg/g FW). The most common change in green plants is the loss of chlorophyll due to senescent tissues, causes a change of color from brilliant green to a wide variety of colors (yellow, brown and orange) (30). Vacuum cooling technology effectively retarded the loss of the chlorophyll contents of baby cos lettuce samples during storage, the similar results was found in comparative precooling of broccoli, vacuum cooling resulted in the maintenance of a chlorophyll content of 11.28 mg/100 g, which was significantly greater than those observed after the cooling water and cold room treatments (71). Moreover, Rennie *et al.* (2001) discussed that the stress due to different pressure reduction rates for vacuum cooling lettuce did not influence to changing of chlorophyll fluorescence during storage, the absence of difference in chlorophyll fluorescence readings suggests that different rates of pressure reduction were not stress the lettuce than in regular vacuum cooling (72).

4.3.3.2 Carotenoid content

Baby cos lettuces precooled with forced-air, vacuum and room cooling were characterized by significantly higher levels of carotenoid content compared to control during cold storage ($p \leq 0.05$) (Table 4.11). At day 9 of storage, forced-air cooled vegetable and control showed the higher level of carotenoid content than those vacuum and room cooling related with the rapidly deterioration of chlorophyll content. Baby cos lettuce, like most green leafy vegetables, is a major source of dietary carotenoids and

chlorophylls which also may have specific dietary activities (90). Kim *et al.* (2016) reported that β-carotene and lutein are the primary carotenoids in lettuce. However, carotenoid content varies with lettuce types which butterhead, romaine, and green and red leaf types showed higher β-carotene and lutein than in crisphead lettuce (91).

4.3.3.3 Total soluble solids

Soluble carbohydrate are often estimated using total soluble (dissolved) solids (TSS) or soluble solid concentration (SSC). Vegetables are low in organic acids with little starch at maturity can have as much as 95% TSS as soluble carbohydrates. However, in vegetables containing starch, fructosan, or other storage carbohydrate having TSS < 5%. Many vegetables contain storage reserves such as fructosans or starch, therefor soluble carbohydrate can be created from degradation of the storage carbohydrate, thus maintaining nearly constant concentration of soluble sugar for a long period of storage (92). Zhan *et al*. (2013) reported the average TSS of lettuce during cold storage range from 4.0-2.6 % depending on several factor such as cultivar, leave positon, chemical treatment, storage condition and storage duration (93). The result indicated that TSS of baby cos lettuce quite constant during low temperature storage period. However, total soluble solid contents in forced-air cooling and room cooling higher than control and vacuum cooling (Table 4.11). The increasing of TSS might be due to a result of reducing water content of sample lead to high concentration of soluble solid accumulated in samples which related to the higher weight loss percentage found in forced-air cooling and room cooling (Table 4.10) At day 13 of storage, the results indicated significant difference of total soluble solids ($p \leq 0.05$) that forced-air cooling and room cooling showed higher total soluble solids than vacuum cooling follow as 3.33, 3.13 and 3.05 %, respectively.

4.3.3.4 Phenolic content

The total phenolic content of baby cos lettuce indicated no significant difference in precooled sample and control during storage after day 1 and day 7 ($p \geq 0.05$) (Table A8). The total phenolic content was observed significant higher ($p \leq 0.05$) in vacuum cooling than forced-air cooling, room cooling and control until day 9 of storage. Vacuum cooling maintained higher total phenolic content than other treatments (Table 4.12). Although there are some report suggested that phenolic compounds of most leafy vegetables are generally stable during cold storage (< 4 °C), some research had also

report losses of polyphenols during the storage of lettuce, which may related to the enzymatic oxidation of phenolic compounds by PPO and POD (94). Our results indicated that vacuum cooling could be able to maintain the phenolic content during storage of baby cos lettuce which related to high level of antioxidant activity. Phenols are very important plant constituents because of their radical scavenging ability due to their hydroxyl groups which act as bioactive potential attributed to antioxidant and antibacterial activities (95) Llorach *et al.* (2001) suggested that, the dominant phenolic acid fractions in the lettuce leaves detected by HPLC analysis were caffeic, chlorogenic, and ferulic acids and derivative of coumaric acid (37).

4.3.3.5 Ascorbic acid

The results indicated a positive effect of vacuum cooling on the ascorbic acid content of baby cos lettuce, and maintained greater ascorbic acid content than other treatments (Table 4.12). After 9 days of storage the results showed the significant difference ($p \leq 0.05$) that vacuum cooling maintained ascorbic acid (8.38 µg/100g FW) which higher than forced-air cooling (5.98 µg/100g FW), room cooling (5.98 µg/100g FW) and control (5.39 µg/100g FW) until day 13 of storage. After 16 days of storage, the ascorbic acid content of baby cos lettuce treated by vacuum cooling was 4.94 mg/100g FW. In terms of the relative dietary contributions of a given sample, it was not only the nutrient concentrations, but also the level of consumption of the food that are important. Therefore, significant differences were observed between precooled and control samples. Nevertheless, the vitamin C content not only depends on varieties, other pre-harvest and postharvest factors can be influencing in the vitamin C content (96). The ascorbic acid levels could decrease in response to high temperature. The same results was found in storage broccoli which simulating temperature abuse from retail organizations to the customer. The experiment conclude that temperature significantly affected ascorbic acid contents which show the greater loss of ascorbic acid correlated to higher storage temperature (97). Temperature management after harvest is the most important factor to maintain vitamin C of fruits and vegetables are at higher temperatures and with longer storage durations accelerated losses of vitamin C. Moreover, conditions favorable to water loss after harvest results in a rapid loss of vitamin C especially in leafy vegetables (96).

4.3.3.6 Antioxidant activity

The antioxidant activity values determined by DPPH assay. The results showed that after cold storage for 9 days, $DPPH^{\bullet}$ scavenging activity of vacuum cooled baby cos lettuces (4.72 µgTrolox/g FW) which were significantly higher ($p \leq 0.05$) forced-air cooling (3.53 µgTrolox/g FW), room cooling (4.02 µgTrolox/g FW) and control (4.05 µgTrolox/g FW).Moreover, vacuum cooling treatment indicated higher level of antioxidant activity than forced-air and room cooling treatment until storage for 13 days (Table 4.12). Antioxidant capacity of vegetables is known to depend on a wide number of compounds and several phytochemicals, such as flavonoids, phenolic acids, amino acids, ascorbic acid, tocopherols and pigments, might contribute to the total antioxidant activity (98). The results of the study of Llorach *et al.* (2001) illustrate that with the DPPH assay showed that baby lettuce and chicory byproduct extracts were those showing the highest activity, followed by romaine and finally iceberg extracts and antioxidant capacity indicated the linearly correlated with the phenolic content (99). The antioxidant activity of vegetable extracts has been correlated to their content of phenolic components, due to their property of scavenging free radicals. Nicolle *et al.* (2004) supported that Antioxidant activity of lettuce is mainly associated with its content of phenolic compounds, vitamins C and E, chlorophyll and carotenoids (100). Moreover, there are collaboration between phenolic antioxidants and vitamin C in term of protect vitamin C from oxidative degradation. Therefore, in this study was found that precooled samples can preserved high level of vitamin c and antioxidant activity during cold storage. Clearly, temperature management is an effective way to delay the loss of nutrients such as antioxidant and vitamin C of postharvest produce and prolong the shelf life. Therefore, it is important to maintain the cold chain and reduce temperature fluctuations in order to maintain qualities and extend the postharvest shelf life (97).

Table 4.11 Chlorophyll content, carotenoid content and total soluble solids of baby cos lettuce during storage at 4 °C with 85 % RH

Storage period	***Treatment***	***Chlorophyll content (µg/g FW)***	***Carotenoid content (µg/g FW)***	***TSS (%)***
0 day	Control	46.46 ± 2.35^{a}	1.56 ± 0.18^{a}	3.13 ± 0.23^{a}

	Forced-air	46.64 ± 4.53[a]	1.61± 0.09[a]	3.20 ± 0.28[a]
	Vacuum	49.37 ± 2.48[a]	1.59 ± 0.05[a]	3.20 ± 0.00[a]
	Room	47.39 ± 2.99[a]	1.61 ± 0.14[a]	3.20 ± 0.00[a]
9 days	Control	33.37 ± 1.28[b]	2.17 ± 0.01[b]	2.97 ± 0.06[a]
	Forced-air	42.75 ± 2.09[a]	1.67 ± 0.13[d]	3.13 ± 0.31[a]
	Vacuum	45.41± 1.79[a]	1.89 ± 0.12[c]	2.95 ± 0.21[a]
	Room	35.95 ± 1.63[b]	2.61 ± 0.12[a]	3.17 ± 0.21[a]
13 days	Forced-air	29.90 ± 0.29[b]	1.67 ± 0.16[a]	3.33 ± 0.06[a]
	Vacuum	32.53 ± 0.84[a]	1.90 ± 0.06[a]	3.05 ± 0.06[b]
	Room	28.56 ± 1.25[b]	1.86 ± 0.21[a]	3.13 ± 0.06[ab]
15 days	Vacuum	26.16 ± 2.12	1.94 ± 0.05	3.33 ± 0.06
16 days	Vacuum	25.82 ± 2.65	1.99 ± 0.09	3.13 ± 0.06

Values designated by the same letter are not significantly different (p > 0.05). Lower case letter are used for comparisons during storage.

Table 4.12 Ascorbic acid, antioxidant activity and total phenolic content of baby cos lettuce during storage at 4 °C with 85 % RH

Storage period	***Treatment***	***Ascorbic acid (µg/100 g FW)***	***Antioxidant activity (µgTrolox/gFW)***	***Total phenolic content mgGAE/g FW***
0 day	Control	9.62 ± 0.02[a]	1.57 ± 0.11[a]	15.15 ± 0.5[a]
	Forced-air	10.26 ± 1.11[a]	1.53 ± 0.09[a]	14.65 ± 0.5[a]
	Vacuum	10.90 ± 1.11[a]	1.54 ± 0.10[a]	14.73 ± 0.34[a]
	Room	8.97 ± 1.11[a]	1.43 ± 0.08[a]	14.74 ± 0.87[a]
9 days	Control	5.39 ± 0.00[b]	4.05 ± 0.40[b]	22.44 ± 0.45[c]
	Forced-air	5.98 ± 1.04[b]	3.53 ± 0.16[b]	21.94 ± 0.78[c]

	Vacuum	8.38 ± 1.04[a]	4.72 ± 0.41[a]	26.61 ± 0.34[a]
	Room	5.98 ± 0.64[b]	4.02 ± 0.38[b]	24.81 ± 0.73[b]
13 days	Forced-air	5.00 ± 0.41[b]	3.72 ± 0.58[a]	20.70 ± 2.15[a]
	Vacuum	6.88 ± 0.27[a]	3.28 ± 0.43[ab]	21.52 ± 1.19[a]
	Room	4.76 ± 0.63[b]	2.52 ± 0.26[b]	21.35 ± 0.62[a]
15 days	Vacuum	6.06 ± 1.05	2.76 ± 0.30	20.70 ± 0.79
16 days	Vacuum	4.94 ± 2.14	2.61 ± 0.26	20.14 ± 0.95

Values designated by the same letter are not significantly different ($p > 0.05$). Lower case letter are used for comparisons during storage.

4.4 Experiment 4: Rapid Determination of Lettuces Antioxidant Capacity by e-Tongue based on Flow Injection Coulometry.

4.4.1 Coularray profile of lettuce extract

The typical raw signals that obtained after injection of a methanolic extracts of lettuce samples into a flow injection system coupled with Coularray detector with 16 sensors, poised at potentials from +100 to +850 mV (vs Pt reference electrode), at step of 50 mV. (Figure 4.40A) The analysis lasts in less than 20 s, resulting in a three dimensional plot, with sixteen current signals plotted as a function of time. Afterwards, the system is left at rest for about 40 s, until the background current returns to a steady value. This implies that the system is able to analysis up to 60 sample h^{-1}. The cumulative peak areas of each current signal was plotted as a function of time, leading to a hydrodynamic voltammogram. (Figure 4.40B) The magnitude of the current intensity of each coulometric sensor reflects the polarizing potential applied. This,

ultimately, reveals the tendency of a sample to get oxidized. In the specific case of lettuce samples, two inflection points are observed, respectively, at +400 and +650 mV (Figure 4.40A and B). Although lettuce extracts are composed by several redox species, the presence of two inflection points reveals that, overall, the oxidation of lettuce samples can be described by two electron transfer processes. The first process is the contribution of those antioxidants showing a facile electron transfer. Accordingly, it expresses the antioxidant power of the sample. This process is separated by about +250 mV from a second oxidation step. The shift of potential between the two oxidation processes may indicate the presence of an overriding chemical reaction, similar to other electrochemical mechanisms that is characteristic of many phenolic compounds (101). Clearly, this second oxidation process can also account for any redox species that was not oxidized before (i.e. having lower antioxidant power).The peak area of single channel poised at +400 mV for increasing concentration of ferrocene methanol (from 0 to 120 µM) showed that peak area is increasing linearly as a function of concentration of ferrocene methanol from 10 to 120 µM (slope=0.95, R^2=0.99), as expected from a faradaic process (Figure 4.40C). The calculated analytical sensitivity is 0.46 with limit of detection 15.5µM. Instead, the results of spiking the lettuce extract with standard solutions of ferrocene methanol (from 0 to 120 µM) by standard addition method (slope=0.93) (Figure 4.40D). This clearly indicates that the presence of the matrix effect is negligible. Also, when the line is extrapolated toward the zero, the resulting concentration leads to an estimate of redox activity contained in the sample, as expressed by equivalent of ferrocene methanol (intercept=24 µC).

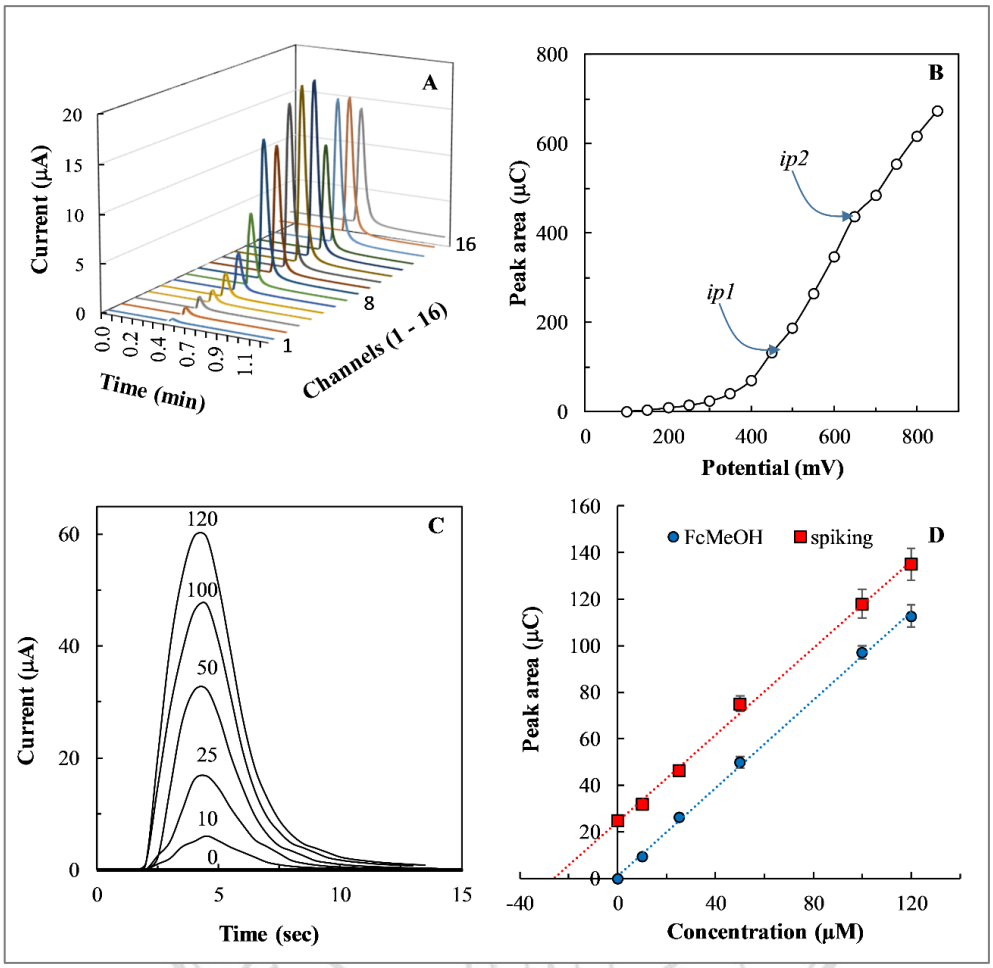

Figure 4.40 (A) Raw data from the lettuce extract injection in 16 channel coulometric array detector poised from 100 to 850 mV, by step of 50 mV. (B) Corresponding hydrodynamic voltammogram where the charge of each channel signal is plotted as a function of the applied potential; (C) raw data of one single channel poised at +400 mV for increasing concentration of ferrocene methanol *(*from 0 to 120 µM*)*; *(*D*)* calibration curve corresponding to the signal shown in (C) and results of a standard addition method where lettuce extract has been fortified with increasing concentration of ferrocene methanol (from 0 to 120 µM*).*

4.4.2 Effect of the drying and solvent extraction

The Coularray detector was next used to compare antioxidant extraction methods from lettuce samples. The treatments consisted of a drying step, followed by solvent

extraction. Drying was perfomed with two different techniques, respectively, liquid nitrogen drying and lyophilization. Instead, the solvent extraction was performed with three different solvents, respectively, (a) methanol, (b) ethanol or (c) acetonitrile. The results showed significant differences between different extracts. The overall charge measured for each of 16 channels of the Coularray detector as a function of different extraction protocols (Figure 4.41). Regardless to the solvent used, the samples that were preliminarily dried with liquid nitrogen have the lowest peak areas at all channels compared to the freeze dried samples. Apparently, the fast cooling achieved with liquid nitrogen impedes the growth of large ice crystals inside the sample, limiting the demage of the tissues (102). This, in turn, reduces the efficiency of the subsequent solvent extraction step. Instead, lyophilization allows the extraction of the higher amount of antioxidants, as results from the highest peak area signals observed. The increase of antioxidant capacity due to freeze drying is also reported by other authors (103).

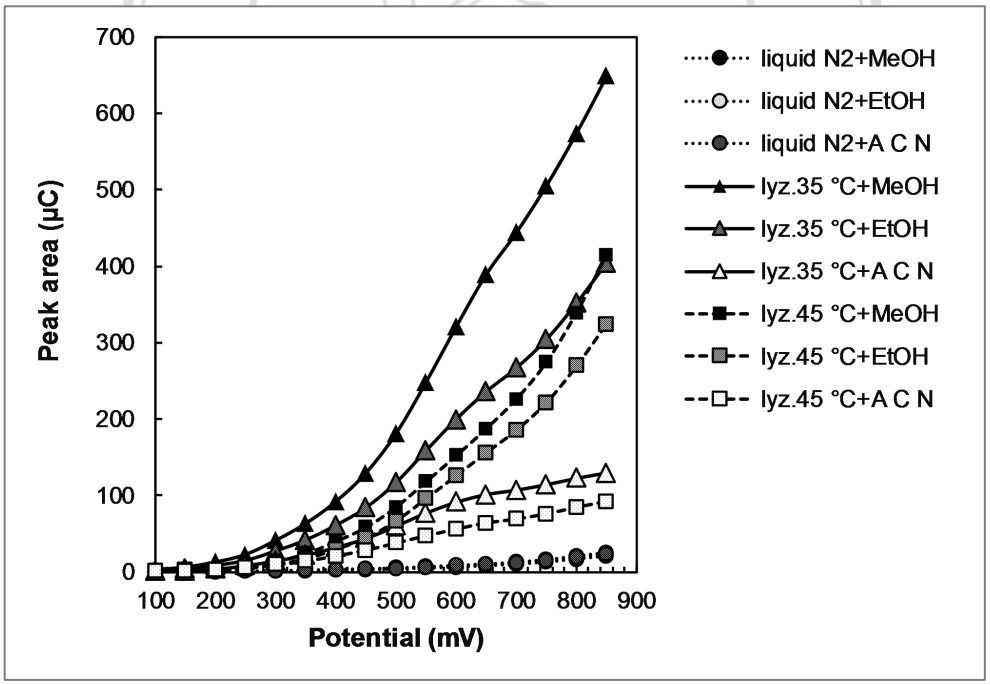

Figure 4.41 Comparison of different extraction techniques. Cumulative peak area *(*µC*)* of 16 channels poised at 100-850 mV is shown.

Accordingly, the results of flow injection analysis by Coularray correlate with the antioxidant capacity measured by traditional DPPH assay (data for the channel +400 mV is shown in Figure 4.42) with $R^2 = 0.98$. Also, previously published studies showed good correlation of electrochemical methods with antioxidant capacity evaluated by

traditional spectrophotometric assays (ABTS and DPPH) (104, 105). Thus, the results suggested that freeze drying allows better extraction of antioxidants from lettuce material.

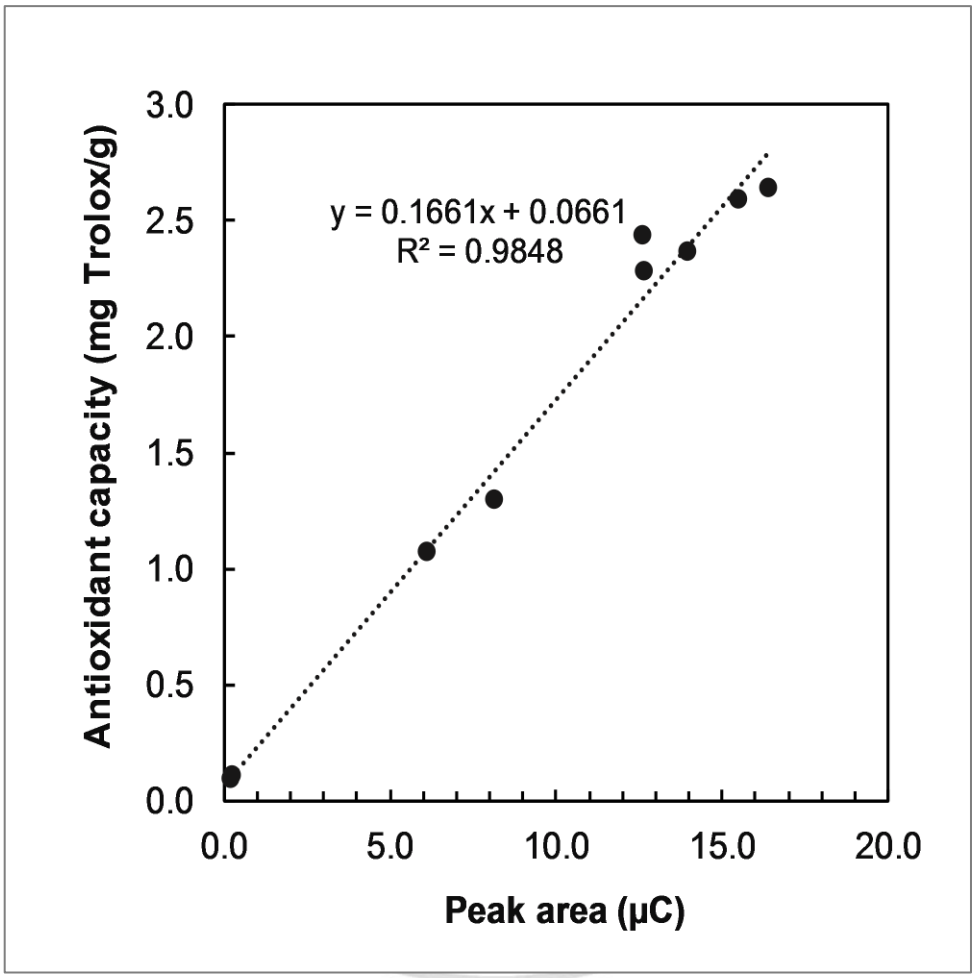

Figure 4.42 Correlation graph of peak area at +400 mV by Coularray flowinjection and antioxidant activity by traditional DPPH scavenging method.

Moreover, among the freeze dried samples, the results indicate a significant variation of the antioxidant capacity depending on the solvent used. In details, two ANOVA with “drying” and “solvent” as fixed factors (Figure 4.43) showed that both of them make a significant influence on the extraction of antioxidant compounds ($p \leq 0.05$, Fischer’s LSD). Compared to acetonitrile extracts, the amount of antioxidants was 1.82 times higher in ethanol and 2.57 times higher in methanol. Different solvents have various capacity to dissolve antioxidant species, as a results of differences in solvent

polarity (106) Usually, the least polar solvents are considered to be suitable for the extraction of lipophilic phenols. The most suitable solvent for extraction of antioxidants from the lettuce was methanol.

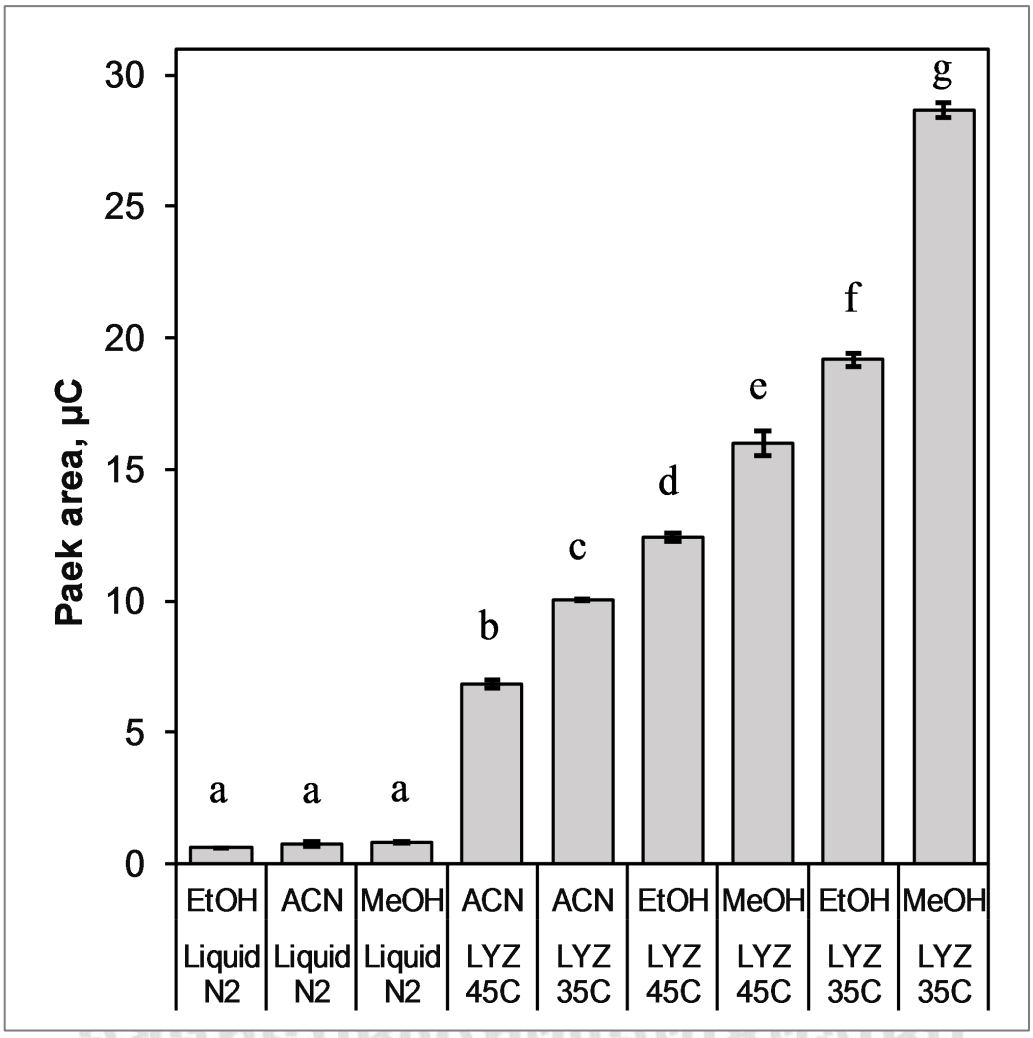

Figure 4.43 Peak area at Coularray channel +400 mV, µC. Values are means of triplicate analyses for each extract. Values for extracts with different letters were significantly different at α=0.05 based on Fischer's method of multiple comparison. Results are presented as means ± SD for triplicate analyses.

Finally, within the freeze dried samples, a significant effect was observed depending on the lasting temperature of freeze drying process. When the second stage of the freeze drying process was conducted at temperature of 35°C, the resulting

antioxidant capacity of the extracts was always higher than those obtained from samples freeze dried up to 45°C. Apparently, the final temperature affects the stability of antioxidants in lettuce. According to the obtained results, freeze drying (35°C) with methanol extraction was chosen for further experiments.

4.4.3 Effect of precooling treatments during storage

The Coularray detector was next used to compare the effect of four storage pre-treatments to preserve the antioxidant activity of the lettuce samples: untreated control, room cooling at 4°C, vacuum cooling (36 min, 5°C) and fast cooling (21 min, 2°C). After each treatment, the samples were stored for 7 days at 5°C. The antioxidant activity, moisture content and color of each sample was measured before treatment and after storage. Vacuum cooling with a final pressure of 5.0 mbar allowed a fast cooling down to 2.10 °C completed in 21 min, which was 11 times faster compared to the room cooling. The results are in agreement with previous studies reporting that vacuum cooling with a final pressure 0.7 kPa resulted about 13 times faster than conventional cooling of iceberg lettuce at 6 °C (27). On the other hand, both vacuum cooling treatments with final pressures of 5.0 and 10.0 mbar showed higher weight loss than room cooling caused by the loss of water due to the rapid evaporation under low pressure during the process.

Coularray analysis showed significant differences between samples after seven days of storage. Antioxidant capacity of samples after fast cooling and vacuum cooling was 20% higher compared to the control (Table 4.13) and room cooling. The results were in agreement with the DPPH assay. Moisture content of room cooled and vacuum cooled lettuce was significantly lower compared to control. Comparing CIELAB color results of control sample before starting the storage ($L^* = 68.05 \pm 1.04$; $a^* = -12.54 \pm 1.26$; $b^* = 27.09 \pm 1.30$), significant color changes after seven days were observed. The values represent the green–red ($-a^* + a^*$), blue–yellow ($-b^* + b^*$) color hue and lightness (L^*) of measured extracts. It was observed that room and vacuum cooling at 10.0 mbar had higher lightness values compared to the control sample and the lettuce after fast cooling. Samples after fast cooling at 5.0 mbar had green-yellow tone, instead the room cooling resulted in color change towards red and blue. This changes may be due to the oxidation process on the surface of the lettuce (Table 4.14).

Table 4.13 Antioxidant capacity and sum of 16 peak areas Coularray (μC) of lettuce treated with different precooling methods. Results are presented as means ± SD for triplicate analyses

Treatments	Sum of 16 peak areas Coularray (μC)	DPPH (mg TAE / g)
Control	637 ± 14 [a]	2.4 ± 0.1 [a]
Room cooling	651 ± 66 [a]	2.3 ± 0.2 [a]
Vacuum cooling	769 ± 14 [b]	2.6 ± 0.2 [b]
Fast cooling	763 ± 44 [b]	2.6 ± 0.1 [b]

Values designated by the same letter are not significantly different (p > 0.05). Lower case letter are used for comparisons during storage.

Table 4.14 Moisture and color parameters of lettuce treated with different precooling methods. Results are presented as means ± SD for triplicate analyses

Treatments	Moisture content (%)	L*	a*	b*
Control	91 ± 1.8 [a]	60.4 ± 0.4 [b]	-17.97 ± 0.9 [b]	33.41 ± 0.34 [bc]
Room cooling	90 ± 0.8 [ab]	67.6 ± 0.8 [a]	-15.56 ± 1.02 [a]	31.90 ± 1.71 [c]
Vacuum cooling	88 ± 0.6 [b]	66.9 ± 0.5 [a]	-16.96 ± 0.8 [ab]	34.97 ± 1.36 [b]
Fast cooling	89 ± 0.4 [b]	59.8 ± 0.6 [b]	-20.32 ± 0.1 [c]	38.35 ± 1.27 [a]

Values designated by the same letter are not significantly different (p > 0.05). Lower case letter are used for comparisons during storage.

The main principal component graph summarizes the results of flow injection analysis by Coularray, DPPH, CIELAB and moisture analysis (Figure 4.44). The first

two principal components retained 83.68 % of the variance, most of which (72.46 %) was accounted by the first principle component F1. The first principal component was loaded mainly with the antioxidant capacity of extracts by Coularray detector and DPPH assay and moisture. The second axis (11.22 %) resulted mostly from small changes in color. Lettuce samples after vacuum cooling and fast cooling are positioned on the positive side of the F1, whereas the control and room cooling samples are positioned on the negative side. Relative positioning of the samples is clearly indicating higher antioxidant capacity of the samples after vacuum and fast cooling compared to the untreated control and the lettuce samples after room cooling. In summary, the proposed electronic tongue based on flow injection with Coularray detector is a fast and sensitive method for screening of antioxidant capacity of fresh lettuce extracts.

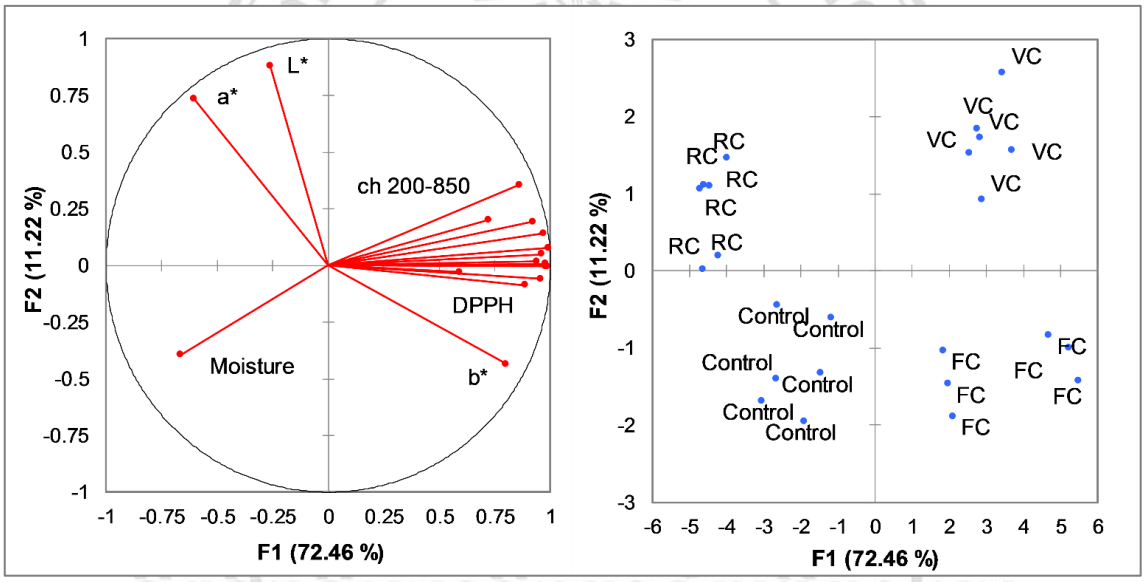

Figure 4.44 Principal Component Analysis of lettuce extracts after various precooling treatments and seven day storage at 5°C *(n=6)*.