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Effects of Dolsan Leaf Mustard Powder on the Quality of Frying Batter

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The anti-oxidant activity, sinigrin content, and sensory evaluation of frying batter treated with Dolsan leaf mustard powder (DLMP) were investigated. These endpoints were measured in the control sample (batter without DLMP) and seven other batters with the addition of DLMP in the following quantities: 0.03 g (S-1), 0.02 g (S-2), 0.06 g (S-3), and 0.3 g (S-4, S-5, S-6, and S-7 with different amounts of red pepper powder). The acid value (AV) and peroxide value (POV) were low in the S-4 and S-5 batters; the total polyphenol content was 190.97 mg GAE/g in the control sample and 721.17 mg GAE/g in S-4; and the total flavonoid content of S-6 was 67.82 mg QE/g which was higher than that of the control sample. The anti-oxidant activity was measured by EDA, ABTS, and FRAP assays and was seen to rise as the amount of DLMP increased. Moreover, sinigrin content was higher in S-4, S-5, S-6, and S-7 than in the control, S-1, S-2, and S-3, and there were no significant differences among S-4 to S-7 when just 0.3 g of DLMP was added. Sensory evaluation showed that color, flavor, taste, texture, and overall acceptability values were significantly higher in S-4 and S-5, and that there was no significant difference across the samples. The addition of DLMP is therefore expected to increase the anti-oxidant activity of frying batter which would be effective in improving the storage and quality of the product.

Key words : Antioxidant activities, Dolsan leaf mustard powder, frying batter, sinigrin

Introduction

With increased economic development and improvements in living standards, the interest a healthy lifestyle and in food with stability and functionality has increased. Furthermore, there is a continuing increase in the demand for plants products that have excellent physiological activity and no side effects. Thus, studies to investigate these natural sources have garnered considerable attention. Dolsan leaf mustard (DLM) (*Brassica juncea*) is one of the leaf and stem vegetables that belongs to the *Cruciferae* family. The seeds from this plant are used as acrid spices because of their pungent taste and unique flavor [21]. Cruciferous vegetables are particularly rich in glucosinolates, phenols, flavonoids, and sulfur compounds [2]. These substances are physiologically active and have pharmacological effects in the body [16]. In addition, leaf mustard is a rich source of minerals because it has an abundance of iron, phosphate, calcium, and potassium [22]. In particular, DLM contains more vitamin A, β-carotene, chlorophyll, and ascorbic acid compared with other leafy vegetables, and these components have antioxidant properties [5, 26]. DLM also contains large amounts of thiosulfates and organosulfur compounds, which reportedly inhibit chemically induced tumors [22]. The volatile sulfur component of glucosinolates, which are typical of sinigrin, are responsible for the unique pungent characteristic of DLM [4]. In other words, when the tissue is injured, myrosinase produces various sulfurous components and related substances [3], which exhibit antibacterial activity against yeast, fungi, and bacteria [4]. In addition, active oxygen species produce a significant number of free radicals, which are destroyed by free radical defense mechanisms in the body; however, *in vivo*, these defense mechanisms and the generation and elimination mechanisms of reactive oxygen species are absent [17, 24]. These diseases are closely related to meat consumption a low fiber diet, and Western-influenced diets. Hence, food and health have become more important to the general public. The use of synthetic medicines and synthetic food additives has been restricted due to safety issues. Consequently, there has been a wide range of studies to identify physiologically active substances in natural products and foods that have no side effects [8, 23]. There is a perception that it is unhealthy, such as feeling when eating

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fried products and rancidity of fats and oils during storage. This study was conducted to investigate the physicochemical properties of Dolsan leaf mustard powder (DLMP) and the quality changes after its addition to frying batter. The sensory quality evaluation was used to determine the proper addition ratio of DLMP and to apply the ratio to new quality frying batter that reflects the health-oriented needs of consumers by suppressing the negative feelings associated with fried products and the rancidity of oils and fats.

Materials and Methods

Materials and reagents

DLM used in this experiment was purchased in January 2019 at Dolsan leaf mustard Farming Association in Yeosu, Jeonnam Province, dried with a vacuum freeze dryer (FDU-8624, TRK, Japan) and then ground in a vacuum bag and stored at -18℃ as a sample. The frying batter was purchased at local markets and made from soft flour, cornstarch, egg white powder, garlic powder, white pepper powder, red pepper powder, DLMP, and syrup. Sinigrin, Folin-Ciocalteu's phenol reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethyl-benzothizoline-6-sulfonic acid) (ABTS), sodium carbonate, gallic acid, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), iron (III) chloride hexahydrate, Iron (II) sulfate heptahydrate ($FeSO_4 \cdot H_2O$), potassium persulfate, ascorbic acid, sodium chloride (NaCl), aluminum nitrate, potassium acetate, quercetin, methanol and ethanol were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Measurement of acid value (AV)

AV was determined by the method followed Kim et al. [11]. An ethanol: ethyl ether (ratio 1:2) mixture of 100 ml was added to the 0.3 g of the sample and shaken well. Four drops of phenolphthalein solution were then added and shaken for 5 minutes. The solution was then titrated with 0.1 N ethanolic potassium hydroxide solution. The end point was determined by the solution turning reddish red for 30 seconds.

AV (acid value) = 5.611 × (T-B) × F/W

T: Optimum consumption of 0.1 N ethanolic potassium hydroxide solution for this test (ml)

B: Optimum consumption of 0.1 N ethanolic potassium hydroxide solution in the blank test (ml)

F: Titer of the 0.1 N-KOH solution used for titration

W: Sample weight (g)

Measurement of peroxide value (POV)

POV was determined by the method followed by Kim et al. [11]. Addition of 30 ml of chloroform: acetic acid (ratio 2:3) mixture and 1 ml of saturated iodine potassium solution to 0.3 g of the sample. The mixture was stirred lightly for 1 minute and then incubated in the dark for 5 minutes. 30 ml of distilled water were added and titrated with 0.01 N sodium thiosulfate solution. The end-point was the loss of starch-associated coloration (colorless), a blank test was performed separately.

POV (meq/kg) = (A-B) × F × 10/S

A: Optimum consumption of 0.01 N sodium thiosulfate solution for this test (ml)

B: Optimum consumption of 0.01 N sodium thiosulfate solution of the blank test (ml)

F: Titer of 0.01 N sodium thiosulfate solution used for titration

S: Sample weight (g)

Measurement of total polyphenol content

The total polyphenol content in frying batter with DLMP added was measured using colorimetry and the Folin-Ciocalteu's phenol reagent [25]. We added 200 μl of Folin-Ciocalteu's phenol reagent and 2.6 ml of distilled water to 200 μl of the sample. The mixture was allowed to react for 6 minutes at room temperature, and then, 2 ml of 7% (w/v) Na_2CO_3 solution was added. The mixture reacted for 90 minutes, and the absorbance was measured using a microplate reader (Infinite F50, Männedorf, Switzerland) at 750 nm. A standard curve was constructed using gallic acid as the standard, and the polyphenol content was reported in mg gallic acid equivalents (GAE)/g.

Measurement of total flavonoid content

The total flavonoid content in the frying batter with DLMP added was measured using the method followed by Moreno [18]. One milliliter of the sample, 0.1 ml of 10% aluminum nitrate, 0.1 ml of 1 M potassium acetate and 4.3 ml of 80% (v/v) ethanol were sequentially added, and the absorbance was measured at 415 nm by allowing the mixture to react in a dark room for 40 minutes. A standard curve was constructed using quercetin as the standard, and the flavonoid content was reported in mg quercetin equivalents (QE)/g.

Measurement of electron donating ability (EDA)

The electron donating ability (EDA), which is the radical scavenging effect of 1,1-diphenyl-2-picrylhydrazyl (DPPH), was shown as the reducing power of the DMS and DLM extracts. We added 0.5 ml of 0.5 mM DPPH solution to a test tube containing 1 ml of the sample, 1 ml of methanol, and 0.99 ml of 100 mM sodium acetate buffer (pH 5.5), and the mixture was agitated and left to react in the dark for 5 minutes. The concentration of the remaining radicals was measured at 517 nm and was reported according to the following equation [27]:

EDA (%) = (1 - absorbance of the solution with the sample added/absorbance of the solution without sample) ×100

2,2-azino-bis(3-ethyl-benzothizoline-6-sulfonic acid) (ABTS) radical scavenging activity

The ABTS radical scavenging activity was measured using a slight modification to the method reported by Kriengsak et al. [13]. Equal volumes of 1.8 mM ABTS solution and 0.63 mM potassium persulfate were combined and left to react in the dark for 24 hr at 37℃ to create an ABTS solution with ABTS free radicals; the solution was modified so that the absorbance at 735 nm was 1.4±0.1. Five ml of the ABTS solution with ABTS radicals was added to 0.1 ml of the sample, and the mixture was allowed to react for 7 minutes; subsequently, the absorbance was measured at 735 nm. The results were calculated according to the following equation:

ABTS radical scavenging activity (%) = (1 - absorbance of the solution with the sample added/absorbance of the solution without sample) ×100

Measurement of ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power (FRAP) was measured using a slight modification of the method reported by Benzie et al. [1]. We mixed 30 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyls-triazine (TPTZ) dissolved in 40 mM HCl, and 20 mM iron (III) chloride hexahydrate in a 10:1:1 (v:v:v) ratio. We mixed 150 µl of the sample with 2,850 µl of the reaction mixture and allowed them to react for 30 minutes; then, the absorbance was measured at 593 nm. A standard curve was constructed using $FeSO_4 \cdot H_2O$, and FRAP content was quantified in mg $FeSO_4$ equivalents/g extract.

Sinigrin standard curve and high-performance liquid chromatography (HPLC) operating conditions

The standard reagent sinigrin was manufactured at 125, 250, 500, and 1,000 ppm; the measurements were repeated three times, and the mean of the three values was used to create a standard curve, which was used to measure the level of sinigrin in frying batter with added DLMP. HPLC was performed using a Shimadzu JP/LC-20Avp, fitted with a UV-VIS detector set at 228 nm. Phenomenex Luna 5 um C18 column (4.6×250 mm) was used with a flow rate of 1.0 ml/min and an injection volume of 10 µl.

Sensory evaluation

Ten trained panelists, who were graduate students at the Chonnam National University, performed the sensory evaluation. The color, flavor, taste, texture and overall acceptability were evaluated using a 9-point scale (1 = very weak, 3 = little weak, 5 = moderate, 7 = little strong, 9 = very strong).

Statistical analysis

All tests and analyses were repeated at least three times. The results are expressed as the mean± standard deviation (SD). One-way analysis of variance (ANOVA) and Duncan's test were used for multiple comparisons using the SPSS version 21.0 (SPSS Institute, Chicago, IL, USA). Differences between values were considered statistically significant in all experiments at $p<0.05$.

Results and Discussion

Acid value (AV) and peroxide value (POV) of frying batter

AV is a measure of the proportion of fat turning rancid; it is obtained by measuring the free fatty acid content formed by hydrolysis of the oil present in the food. The production of free fatty acids promotes auto-oxidation, which degrades the quality of the product [21] and has a negative effect on its sensory properties. The different ratios of DLMP added to the frying batter are shown in Table 1. The AV and POV of the control group and the seven samples are shown in Table 2. The AV of the control group containing 0 g of DLMP with 0.06 g of red pepper powder was the highest value at 0.62 among all the samples, and frying batter (S-4) to which 0.3 g of DLMP and 0.06 g of red pepper powder were added had an AV of 0. This finding is consistent with the

Table 1. Frying batter ingredients and the amounts of Dolsan leaf mustard powder added

Ingredients (g)	Control	S-1	S-2	S-3	S-4	S-5	S-6	S-7
Soft flour	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Corn starch	4.34	4.34	4.34	4.34	4.34	4.34	4.34	4.34
Egg white powder	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Garlic powder	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
White pepper powder	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Red pepper powder	0.06	0.03	0.02	0	0.06	0.03	0.02	0
Dolsan leaf mustard powder	0	0.03	0.04	0.06	0.3	0.3	0.3	0.3
Syrup	36.0	36.0	36.0	36.0	36.0	36.0	36.0	36.0
Total	61.2				61.5			

AV of red ginseng powder or mugwort samples, which was low in proportion to their concentrations [7, 12]. POVs were 3.21-5.78 meq/kg for the control group, S-1, and S-2, and 0.11-0.54 meq/kg or S-4, S-5, S-6, and S-7 containing 0.3 g DLMP. AV and POV inhibited rancidity with increasing DLMP content, and significantly differed from the control group [6].

Total polyphenol and total flavonoid contents of frying batter

Phenolic compounds are one class of widely distributed plant secondary metabolites. They have various structures and molecular weights. The phenolic hydroxyl (OH) groups of these compounds enable them to be easily combined with proteins or other giant molecules. They exhibit many physiological activities such as antioxidant or anticancer activities. Flavonoids have a diphenylpropane (C6-C3-C6) skeletal structure and are widely distributed in vegetables, fruits, seeds, and nuts [9]. The total polyphenol and total flavonoid content of frying batter with different DLMP additions are shown in Table 3. The total polyphenol and total flavonoid contents were significantly higher than those in the control group without DLMP, indicating a significant increase with increasing DLMP concentration. In particular, total polyphenol and total flavonoid contents were about three times higher in samples, S-4, S-5, S-6, and S-7 than in S-2, S-3 and S-4 [10, 28], but there were no significant differences between the samples. Generally, active ingredients including total phenolic compounds in natural plants are known to have a larger amount of residue than that of hot air drying in vacuum freeze drying [12]. As a result, total polyphenol and total flavonoid content are expected to have a positive effect on antioxidant activities.

Table 2. Acid value (AV) and peroxide value (POV) of frying batter with Dolsan leaf mustard powder added

Samples[1)]	Acid value (AV) (KOH mg/g)	Peroxide value (POV) (meq/kg)
Control	$0.62\pm0.22^{C*}$	5.78 ± 0.67^{D}
S-1	0.31 ± 0.11^{C}	4.71 ± 0.19^{C}
S-2	0.19 ± 0.19^{B}	3.96 ± 0.56^{B}
S-3	0.12 ± 0.11^{B}	3.21 ± 0.37^{B}
S-4	0.00 ± 0.00^{A}	0.21 ± 0.19^{A}
S-5	0.06 ± 0.11^{A}	0.11 ± 0.00^{A}
S-6	0.12 ± 0.11^{B}	0.54 ± 0.19^{A}
S-7	0.19 ± 0.19^{A}	0.32 ± 0.37^{A}

[1)]Refer to Table 1.

* Data represent the mean ± SD of experiments performed in triplicates. The different lower-case letters (superscript) in the same column (A-D) indicate the significant difference by Duncan's multiple range test ($p<0.05$).

Antioxidant activities and sinigrin content

The antioxidant activity and sinigrin contents of frying batter with different DLMP additions using EDA, ABTS and

Table 3. Total polyphenol and total flavonoids content of frying batter with Dolsan leaf mustard powder added

Samples[1)]	Total polyphenol (mg GAE/g)	Total flavonoids (mg QE/g)
Control	$190.97\pm6.69^{b*}$	25.63 ± 0.07^{a}
S-1	227.23 ± 5.29^{b}	26.34 ± 0.03^{a}
S-2	223.78 ± 2.16^{b}	27.00 ± 0.09^{a}
S-3	230.62 ± 2.18^{b}	27.56 ± 0.10^{a}
S-4	721.17 ± 0.39^{c}	67.27 ± 0.05^{c}
S-5	718.11 ± 0.55^{c}	66.61 ± 0.05^{c}
S-6	713.26 ± 0.66^{c}	67.82 ± 1.08^{c}
S-7	696.33 ± 0.81^{c}	61.79 ± 0.07^{c}
0.02% Ascorbic acid	97.51 ± 0.31^{a}	50.73 ± 0.00^{b}

[1)]Refer to Table 1.

* Data represent the mean ± SD of experiments performed in triplicates. The different lower-case letters (superscript) in the same row (a-c) indicate the significant difference by Duncan's multiple range test ($p<0.05$).

FRAP method are shown in Table 4. The EDA, ABTS radical scavenging activity and FRAP content significantly increased in S-1, 2, and 3 with increasing amounts of DLMP. The antioxidant activity measured by the FRAP method was generally similar to the result of the antioxidant activity measured by the ABTS method. The sinigrin content, a major component of DLM, was also significantly increased according to the addition. S-4, S-5, S-6, and S-7 which differed in the amount of red pepper powder added to 0.3 g of DLMP, showed high antioxidant activity and sinigrin content with no significant differences among the samples. The antioxidant activity and sinigrin content of the control group were also reported to be altered by the addition of garlic powder, white pepper and red pepper powder to the batter, and were similar to the results of this experiment [14]. Frying batter with high sinigrin content effectively eliminated radicals and had a high antioxidant capacity, while the frying batter with low sinigrin content showed relatively low radical scavenging ability. The results of this study showed that, the addition of DLMP significantly increased antioxidant activity and sinigrin content compared with batter without DLMP addition [19]. When the batter is fried and applied to processed meat products, it is considered to be effective in increasing the antioxidant activity of the product and this increase is thought to correlate with DLMP antioxidant capacity and sinigrin content.

Sensory evaluation

The results of sensory evaluations frying batter with different DLMP additions are shown in Table 5. The color intensity increased with increasing amounts of DLMP compared with that in the control sample, with the highest score of 7.4 in S-4. The flavor and taste values were highest in S-4 and S-5 at 7.0 and 6.2, respectively, and significantly lower in S-1, S-2, and S-3. Taste, texture, and overall acceptability were also better in S-4 and S-5, but the difference was not significant. Although there was no significant difference in the sensory evaluation results with added spices, such as green tea, rosemary, and parsley, it was reported that products that had green tea in them tasted good [15].

Table 4. Antioxidant activities and sinigrin content of frying batter with Dolsan leaf mustard powder added

Samples[1)]	EDA (%)	ABTS (%)	FRAP (mg $FeSO_4$ eq./g)	Sinigrin (mg/100 g)
Control	25.56±0.34^{a*}	13.87±0.01^{a}	250.52±13.18^{c}	13.33±0.02^{a}
S-1	44.14±0.01^{b}	36.46±1.03^{b}	116.32±6.70^{b}	59.00±0.01^{a}
S-2	45.72±0.04^{b}	45.14±0.25^{b}	99.55±71.84^{b}	60.00±0.02^{a}
S-3	58.89±0.01^{b}	59.47±0.57^{b}	161.52±60.89^{c}	58.33±0.03^{a}
S-4	81.12±0.14^{c}	70.14±1.17^{c}	554.84±0.14^{d}	888.00±0.02^{b}
S-5	80.38±0.65^{c}	69.74±0.06^{c}	549.19±0.08^{d}	886.67±0.05^{b}
S-6	80.23±0.15^{c}	65.83±0.96^{c}	543.34±1.99^{d}	880.67±0.03^{b}
S-7	77.97±0.08^{c}	68.49±0.01^{c}	521.77±4.78^{d}	874.00±0.06^{b}
0.02% Ascorbic acid	93.06±0.76^{d}	98.96±0.02^{d}	71.33±4.28^{a}	

[1)]Refer to Table 1.

* Data represent the mean ± SD of experiments performed in triplicates. The different lower-case letters (superscript) in the same row (a-d) indicate the significant difference by Duncan's multiple range test (p<0.05).

Table 5. Sensory properties of frying batter with Dolsan leaf mustard powder added

Samples[1)]	Color	Flavor	Taste	Texture	Overall acceptability
Control	1.40±0.89^{bA*}	1.00±0.00^{aA}	1.00±0.00^{aA}	3.40±1.67^{dA}	2.20±1.79^{cA}
S-1	2.20±1.10^{aA}	2.20±1.10^{aA}	3.00±1.41^{bB}	3.00±1.41^{bA}	3.00±1.41^{bB}
S-2	3.40±0.89^{aB}	3.40±0.89^{aB}	3.80±1.10^{bB}	3.40±0.89^{aA}	3.40±0.89^{aB}
S-3	5.00±0.00^{bC}	3.80±1.79^{aB}	3.40±0.89^{aB}	3.40±0.89^{aA}	3.40±0.89^{aB}
S-4	7.40±0.89^{cD}	7.00±1.41^{cD}	6.20±1.10^{bD}	5.80±1.10^{aB}	7.00±1.41^{cD}
S-5	7.00±0.00^{cD}	7.00±0.00^{cD}	6.20±1.10^{bD}	5.80±1.10^{aB}	7.00±0.00^{cD}
S-6	6.60±0.89^{cD}	5.40±0.89^{aC}	5.80±1.10^{bC}	5.00±0.00^{aB}	5.80±1.10^{bC}
S-7	5.80±1.10^{bC}	5.80±1.10^{bC}	5.40±0.89^{aC}	5.00±0.00^{aB}	6.60±0.89^{cC}

[1)]Refer to Table 1.

* Data represent the mean ± SD of experiments performed in triplicates. The different lower-case letters (superscript) in the same row (a-d) and column (A-D) indicate the significant difference by Duncan's multiple range test (p<0.05).

These results suggest that the addition of DLMP to frying batter may be desirable because of its high antioxidant activity and preference. However, further studies are needed to improve the storage stability of the product.

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초록 : 돌산갓 분말 첨가가 튀김반죽의 품질에 미치는 영향

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돌산갓 분말을 첨가한 튀김반죽의 항산화 활성, sinigrin함량 및 관능검사를 측정하였다. 돌산갓 분말을 첨가하지 않은 대조군과 돌산갓 분말을 0.03 g (S-1), 0.02 g (S-2), 0.06 g (S-3)과 돌산갓 분말0.3g에 고추 가루의 첨가량을 달리하여(S-4, 5, 6, 7) 튀김반죽을 만들었다. 튀김반죽의 산가와 과산화물가는 S-4와 S-5에서 낮은 값을 나타냈다. 총 폴리페놀함량은 대조군에서 190.97 mg GAE/g, S-4에서 721.17 mg GAE/g, 총 플라보노이드 함량은 S-6에서 67.82 mg QE/g으로 대조군보다 높은 함량을 나타냈다. 전자공여능, ABTS 라디컬 소거능과 FRAP로 측정한 항산화 활성은 돌산갓 분말 첨가량이 증가될수록 항산화 활성도 증가함을 나타냈다. 또한 sinigrin 함량은 대조군을 제외하고 S-1, 2, 3보다 S-4, 5, 6, 7에서 높게 나타났으며, S-4, 5, 6, 7은 서로 유의적인 차이는 없었다. 관능검사는 색, 향, 맛, 촉감과 전반적인 기호도는 S-4와 S-5에서 유의적으로 높았으며, 나머지 시료들은 유의적 차이가 없었다. 이로 보아 튀김반죽에 돌산갓 분말의 첨가는 튀김반죽의 항산화 활성을 증가시킴으로써 제품의 저장성과 품질향상에 효과적일 것으로 예상된다.