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The Effect of Different Deheating Processes on Residual Myrosinase Activity, Antimicrobial Properties and Total Phenolic Contents of Yellow Mustard (*Sinapis alba*)

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ABSTRACT: Mustard is a natural multi-functional additive with strong hot flavor. In this research, powdered yellow mustard (*Sinapis alba*) was heated by 21 different thermal processes, statistically designed by Response Surface Methodology to evaluate the effects of combinations of heating media (hot air, hot water and steam) and heating time (0-20 min) on residual myrosinase activity, the minimum inhibitory concentration (MIC), color characteristic and also the major phenolic constituents of each treated sample. The optimum process, for the desirable function was 5 min of heating by hot air. Under optimal conditions, myrosinase activity was reduced by 44%, MIC and lightness (L*) were approximately unchanged, redness (a*) and yellowness (b*) were increased as compared to the control. In spite of the fact that the total phenolic content were reduced severely (>90%) due to deheating processes, but the chromatography spectrum indicated that some compound (caffeic acid) were newly formed or existed in higher concentration (Sinapine) particularary in steam treated samples.

Keywords: *Phenolic Component, Thermal Heating, Yellow Mustard.*

Introduction

Cruciferous vegetables are vegetables of the family Brassicaceae (also called Cruciferae). The family contains over 330 genera and about 3,700 species such as cabbage, broccoli, cauliflower, turnip, rapeseed, mustard, radish, horseradish, wasabi and watercress. Mustard is the third most commonly consumed spice, after salt and pepper. In the second half of the twentieth century, world usage of mustard more than doubled, from 75,000 tons to over 170,000 tons and it becomes more popular spice in fast-food restaurants. It is usually found in three types: yellow or white mustard (*Sinapis alba*), brown mustard (*Sinapis nigra*) and oriental (*Sinapis* juncea) (Smith & Hong, 2003). White mustard is the most widely used- type of mustard in food industry and contains valuable active components including isothiocyanates, complex polysaccharides (mucilagenous substance), phenolic compounds, dithiolthiones & dietary fibers. Sinalbin is a thiocyanate glycoside that exists in white mustard, which is hydrolyzed by myrosinase

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to form a flavor- active Isothiocyanate. Isothiocyanates is responsible for the known "hot" flavor of white mustard and also its antimicrobial and preservative function (Lin *et al.,* 2000). Glucosinolate and/or isothiocyanates may display diverse and interesting biological properties. Some are hepatotoxic or goitrogenic, whereas others have anti-bacterial, anti-fungal, anti-protozoal, nematocidal and or anti-carcinogenic activities (Herzallah & Holley, 2012). Thiocyanate and oxazolidinethione anions are known to compete with iodine in two ways, by inhibiting its uptake through competition with the sodium-iodide symporter (the common term for combined transporters), and binding of iodine to tyrosine residues of thyroglobulin at high concentrations. Hence one of the common symptoms of glucosinolate and /or isothiocyanate exposure is the impairment of the thyroid function, resulting in a hypertrophy of this endocrine gland (goiter). The toxicity of glucosinolate is generally attributed to the isothiocyanates, thiocyanate, oxazolidinethione and nitriles, originating from enzymatic cleavage of glucosinolates by myrosinase. In addition to the above mentioned negative aspects of myrosinase activity, it has also positive functions due to antimicrobial activities. The mechanism of this antimicrobial action is uncertain, and different theories have been approved. Turgis *et al*. (2009), suggested that the antibacterial activity of mustard powder is probably due to the ability of its components (i.e. isothiocyanates) to damage the microorganism cell membrane, lysing and releasing of the outer membrane-associated materials especially ATP from the cells. Investigations of Nilson & Holley, (2012), showed that isothiocyanates may bind to the sulfhydryl groups of microorganism cell enzymes and interfere in its functions. Another valuable component of mustard is phenolic compounds which are main source of antioxidant activity through acting as chain breakers and free radical scavenger (Shahidi *et al.,* 1995). Unfortunately this activity may be severely loss during different processing (Amin & Yee Lee, 2005).

The main objective of this research was to apply 21 different statistically designed thermal processes on white mustard powder and evaluate the positive effects (myrosinase partial inactivation: hot flavor and toxicity reduction) and also the negative effects (loss of antimicrobial and the total phenol contents) of each thermal treatment and finally determine the optimum deheating condition by statistical analysis through response surface methodology (RSM) and desirability function.

Materials and Methods

- *Materials*

Yellow mustard powder (code no: 201) were provided by Pars Siavashan, the local agent of G.S.DUNN (Ontario, Canada). Four microorganisms including *Bacillus subtilis* (PTCC 1254), *Bacillus coagulans* (NBRC 12583), *Saccharomyces cerevisiae* (PTCC 5269) and *Zygosaccharomyces rouxi* (PTCC 5206) were provided by Iranian Research Organization for Science and Technology (IROST). Sabrose Dextrose agar (SDA) and Nutrient agar (NA) (Merck KGaA) were used as growth media for yeasts and bacteria, respectively. Glucose Assay Kit was provided by Zist-Chime (Tehran, Iran)

- *Methods*

- *Heating processes*

Mustard powder was spread in a thin (2cm) layer in an aluminum pan. Three different thermal enzyme inactivation media, hot air, hot water and steam, have been used by heat processing of the samples in oven, water bath and autoclave, respectively. All of the processes applied at the same temperature (100±5 °C) for (0 -20 min) (Eapen *et al*., 1968; Herzallah & Holley, 2012). Different combinations of heating

time and methods were designed by response surface methodologies, Design-Expert 7.0.0 software (Table 1).

- *Residual myrosinase activity*

10 g of the heated mustard powder was homogenized in 90 ml distilled cold water. The homogenate was then centrifuged at 3000g for 15 min at 4°C (Wang *et al,* 1994). Glucose assay kit was used to determine the residual myrosinase activity in supernatant according to the amount of released glucose by UV spectrophotometer at 500nm (Lin *et al.,* 2000).

- *Minimum inhibitory concentration*

Antimicrobial activity of the heated mustard samples were studied according to their minimum inhibitory concentrations (MIC). Surface culture was accomplished in the specific culture media for the selected yeasts and bacteria, and then 4 holes were created in each plate. Suspensions of natural mustard powder and the heated mustard samples were prepared with different concentrations (0.5, 1, 1.5, 2, 2.5, 3, and 3.5 %) and injected in the holes. The plates were incubated for 24- 48 h at 37 °C, and then the minimum inhibitory concentrations of the treated and untreated samples on the microorganisms were determined (Saadoun *et al.,* 2007).

- *Color characteristics*

Color of the heated samples were studied by CS 2000 spectroradiometer (Konica-Minolta, Japan) at 380 -780 nm using D65 illuminant. Color characteristics of each treated sample were described in L*, a*, and b*values which are coordinates in visual uniform color space and are related to x, y, z tristimulus values by the following equations:

$$L^* = 116 (Y / Yn) 1/3 - 16$$

$$a^* = 500 [(X / Xn) 1/3 - (Y / Yn) 1/3]$$

$$b^* = 200[(Y / Yn) 1/3 - (Z / Z n) 1/3]$$

These factors have been specified by the International Committee of Illumination and were calculated by the software system (CS-S10W).

- *Total concentration of phenolic compounds*

Phenolic compounds have been directly extracted from the heated samples (2 gr) by means of 5 ml methanol/water 80:20 (V/V) solution. The suspensions were homogenized in the ultrasonic bath for 15 min at room temperature, and then centrifuged at 5000 g for 25 min. An aliquot of the supernatant was filtered and analyzed by HPLC (Young Lin Acme 2000) according to International Olive Council (2009).

- *Statistical analysis*

Statistically the design of thermal treatments was accomplished by response surface methodologies, Design-Expert 7.0.0 software and one- way analysis of variance (ANOVA) ($p \leq 0.05$) was applied to analyse the results obtained.

Table 1. Different heating processes statistically designed by RSM method

Run	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Heating Media	Steam	Hot air	Hot water	Hot water	Hot air	Steam	Steam	Hot air	Steam	Hot air	Hot air
Heating Time (min)	5	20	12.5	20	5	16.25	12.5	5	5	12.5	16.25
Run	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	
Heating Media	Hot water	Hot water	Hot air	Hot water	Steam	Hot air	Steam	Hot water	Steam	Hot water	
Heating Time (min)	5	5	20	16.25	20	8.75	20	8.75	8.75	20	

Results and Discussion

- *Residual myrosinase activity*

The results of the residual enzymatic activity of the natural mustard powder and different treated samples are shown in Table 2. All of the samples showed a decreasing pattern of enzymatic activity with increasing time. The most and the least effective heating media were steam and hot water, respectively. This might be expected, because of higher heating transfer rate of steam (6000-15000W/m^{2}°C) as compared to hot water (1000-6000 W/m2°C) (Kreith, 2000).

Although heat transfer coefficient for hot water is substantially higher than hot air (10-100 W/m^{2}°C), but the results of this research showed lesser effect on the enzyme inactivation. It seems that forced air circulation in laboratory oven (Memmert universal oven, model UF55) might increase the heating rate of hot air medium and results in reducing the enzyme activity more severely as compared to still hot water method (Yousefi *et al.,* 2014). In the beginning stages (up to 5 minutes) of heating by hot water, an increasing pattern of enzyme activity (from 934.20 to 1152.24 µg glucose released / min) might be observed (Table 2). The same result has been reported by Diogo, 2010 for myrosinase inactivation in broccoli (*Brassica oleracea var. Italica*). It has been reported that myrosinase activity of the low moisture content (<13%) broccoli increased at the moderate temperatures (40-60° C) and decreased by further heating up to the higher

Table 2. Residual enzyme activities of different heat-treated mustard powder samples

Heating media	Heating time (min)	Residual enzymatic activity (µg glucose released / min)	Enzymatic activity reduction (%)
Steam	0	934.20±15.23^{b}	
	5	657.02±11.70 ^{c}	29
	8.75	516.7±20.21^{e}	44
	12.5	411.99±15.01^{fg}	55
	16.75	198.41±13.90 ^{i}	78
	20	101.80±10.01 ^{j}	89
Hot water	0	934.20±15.23^{b}	
	5	1157.74±7.00 ^{a}	-23
	8.75	948.02±11.90^{b}	-1
	12.5	594.5±9.70 ^{d}	36
	16.75	409.67±3.42 ^{fg}	56
	20	388.13±13.02^{g}	58
Hot air	0	934.20±15.23^{b}	
	5	533.54±5.70 ^{e}	42
	8.75	443.04±9.21 ^{f}	52
	12.5	374.91±7.51 ^{g}	59
	16.75	296.32±7.01 ^{h}	68
	20	223.07±8.80 ^{i}	76

temperatures. Therefore it seems that because of lower heating rate of hot water, mustard powder might be heated slowly in this method that provide an opportunity for activating the enzyme at the initial lower temperatures. Wang *et al*. (1994) studied the heating rate of fruits in exposure to hot air and hot water and reported that the circulation rate of the heating medium is an effective factor but it is more important for hot air processes. Therefore this parameter might be the reason of the steeper slope for hot air blanching as compared to hot water, in spite of its lower heat transfer coefficient. Eapen *et al.* (1968) investigated on thermal inactivation of myrosinase from canola seeds and showed that the residual activity was more than 70% after oven heating for 30 min at 105°C. According to the results of Table 2, the inactivation rate was much higher in the present research therefore myrosinase of mustard powder retained its activity about 25% after being heated in the oven for 20 min. Therefore source of the enzyme is an important factor that might cause differences in thermal resistance of the enzyme and also in thermal conductivity and/or internal heat resistance of the product. This result is in agreement with the report by Ghawi *et al.* (2012) who observed lower heat stability of myrosinase in mustard than in canola. Another main reason for this difference is related to the particle size of the product (Wang *et al.*, 1994) that leads to slower heat penetration rate into the particles of whole canola seeds as compared to mustard fine powder.

- *Minimum inhibitory concentrations (MICs)*

The MICs of different treated mustard samples obtained for *Bacillus subtilis*, *Bacillus coagulans*, *Saccharomyces cerevisiae* and *Zygosaccharomyces rouxi* are given in Table 3.

Table 3. Minimum Inhibitory Concentration (%) for different treated mustard samples[*]

Run	Microorganisms species			
	Bacillus subtilis	***Bacillus coagulans***	***Saccharomyces cerevisiae***	***Zygosaccharomyces rouxi***
1	-[†]	-	1.5	3
2	-	-	3	-
3	-	-	1.5	3.5
4	-	-	2	-
5	-	-	1.5	3.5
6	-	-	3	-
7	-	-	2	-
8	-	-	1.5	3.5
9	-	-	1.5	3
10	-	-	2	-
11	-	-	2.5	-
12	3.5	3.5	0.5	2
13	3.5	3.5	0.5	2
14	-	-	3	-
15	-	-	2	-
16	-	-	-	-
17	-	-	1.5	3.5
18	-	-	-	-
19	3	3.5	1	2.5
20	-	-	1.5	3.5
21	-	-	2	-

[*]MIC for the untreated mustard powder against the above mentioned microorganisms were as follows: *Bacillus subtilis* (-), *Bacillus coagulans* (-), *Saccharomyces cerevisiae* (1%), *Zygosaccharomyces rouxi* (3%).
[†]without inhibitory effect (MIC>3.5%).

As it may be observed, only three of the samples (Runs 12, 13 and 19) showed antimicrobial activity against bacterial strains with MICs 3 and 3.5% for *Bacillus subtilis* and *Bacillus coagulans*, respectively, whereas yeasts were more sensitive and inhibited with lower concentrations of mustard (0.5-3%). Shofran *et al*. (1998), and Nilson & Holley, (2012) studied the antimicrobial effect of mustard on different microorganisms and reported higher activity against yeasts as compared to the bacterial species. Lengthening the heating time (from 5 to 20min) of each method (hot air, steam or hot water) resulted in severe degradation of antimicrobial compounds and therefore higher inhibitory effect was obtained. The results of antimicrobial activity (Table 3) and remained enzymatic activity (Table 2) confirm each other, therfore deheated mustard samples with the same amount of residual enzymatic activity (such as runs 7, 10 and 15 where 400 µg glucose / min was released) showed equal antimicrobial activity against the same strain (i.e. *Saccharomyces cerevisiae* with MIC=2%). This might be expected because the antimicrobial activity of the samples is actually related to the amount of benzyl isothiocyanate and this compound is the product of enzymatic degradation of glucosinolates. Therefore by decreasing the residual enzymatic activity or, in other words, by lowering the amount of benzyl isothiocyanate, the antimicrobial activity will be reduced. The same pattern might be observed among other samples such as runs 12, 13 and 19. This result is in agreement with Luciano *et al*. (2011) who investigated the antimicrobial effect of both hot (natural enzyme) and cold (reduced enzyme activity) mustard on *E.coli* and reported that by heating mustard and reducing its enzyme activity, the antimicrobial effect might be decreased. Lara –Lledo *et al*. (2012) studied the antimicrobial activity of mustard powder and sinigrin, and approved that mustard powder is more active than sinigrin because of presence of myrosinase in mustard. According to the results of Table 3, higher concentrations of the treated samples (MICs) might be necessary to inactivate *Zygosaccharomyces rouxi* than *Saccharomyces cerevisiae*. It means the latter yeast is more sensitive. This result is in agreement with the investigation of Shofran *et al*. (1998).

- *Color characteristics*

Values of three main color characteristics (L*, a* and b*) of 21 treated mustard samples are presented in Table 4.

All of the heating methods resulted in changing the color of the samples significantly and made them darker as compared to the control. Among different heating media, steam showed the highest effect on color quality of mustard and caused approximately 19% decrease in L* and 1.8% increase in b* after 20min heating of mustard powder. However data of the redness (a*) of the samples were very interesting. Therefore this parameter in runs 16, 18 is about 6 times greater than the control. These results confirmed more intense heating by steam. It seems that decreasing L* and increasing a* and b* during heating might be the result of undergoing of millard reactions in the presence of mustard proteinaceous compounds (24.94% (dwb)) and carbohydrates (39.49% (dwb)) and producing brown nitrogen-containing high molecular weight pigments, melanoidins (USDA, 1976; Bastos *et al.*, 2012).

- *Total phenolic contents*

The total phenolic contents of heat-treated samples of mustard powder have been shown in Table 5.

Table 4. Color parameters of treated mustard samples*

Run	a*	b*	L*
1	3.04±0.15 e	34.41±0.01 ij	67.82±1.28 defg
2	1.08±0.06 hi	35.35±0.03 f	72.04±0.42 abcde
3	2.03±0.03 g	36.65±0.01 ab	66.02±0.86 fgh
4	3.24±0.01 e	36.72±0.02 a	63.18±0.08 ghi
5	-0.61±0.07 m	34.14±0.06 kl	74.39±0.67 a
6	5.15±0.02 b	35.48±0.03 e	63.54±0.75 ghi
7	4.3±0.00 c	34.53±0.03 i	65.61±0.75 fgh
8	-0.61±0.07 m	34.08±0.02 l	74.28±0.29 a
9	3.04±0.15 e	34.14±0.06 kl	68.04±3.02 defg
10	-0.31±0.01 l	34.71±0.02 h	73.21±0.05 abc
11	-0.03±0.00 k	35.07±0.07 g	72.78±0.09 abcd
12	0.87±0.00 ij	36.39±0.01 c	68.81±2.39 cdef
13	0.89±0.00 ig	36.39±0.01 c	69.1±0.84 bcdef
14	1.08±0.06 hi	35.36±0.01 ef	72.31±1.21 abcd
15	2.42±0.01 f	36.67±0.01 ab	64.83±0.45 fgh
16	6.18±0.01 a	35.67±0.02 d	59.49±1.86 i
17	-0.55±0.01 m	34.35±0.00 k	73.84±1.54 ab
18	6.19±0.00 a	35.67±0.02 d	61.25±1.13 hi
19	1.19±0.02 h	36.56±0.00 b	67.74±1.42 defg
20	3.6±0.01 d	34.59±0.02 j	67.12±1.35 efg
21	3.22±0.01 e	35.46±0.02 a	63.24±0.75 ghi

* Color properties (a*, b* and L*) for untreated mustard powder were -0.85, 34.02 and 74.34, respectively.

Table 5. Total phenolic contents of different heat-treated mustard powder samples

Heating media	Heating time (min)	Total phenolic contents (mg/kg)	Phenolic contents reduction (%)
Steam	0	55455.61±22.1 a	–
	5	4962.35±44.0 cd	91.01
	8.75	4688.22±25.4 e	91.54
	12.5	4584.16±17.5 e	91.70
	16.75	4431.49±5.7 fg	92.02
	20	4351.35±2.4 g	92.15
Hot water	0	55455.61±22.1 a	–
	5	5421.04±12.8 b	90.22
	8.75	5324.01±31.9 b	90.03
	12.5	5058.28±3.1 cd	90.87
	16.75	4922.17±17.0 d	91.12
	20	4621.91±16.4 e	91.66
Hot air	0	55455.61±22.1 a	–
	5	5358.22±22.7 b	90.33
	8.75	5090.95±14.0 c	90.82
	12.5	4732.43±112.6 e	91.46
	16.75	4629.36±17.2 e	91.56
	20	4561.23±67.5 ef	91.77

Untreated mustard powder had the highest content of phenolic compounds (55455.61 mg/kg). Mustard seed is usually considered as a rich source of polyphenolic compounds (37000 - 46000 mg/kg) among different cultivars (Amin & Yee Lee, 2005; Ildiko *et al.,* 2006). As might be expected, in all of the blanching methods, a reduction in

the total phenolic contents of heat –treated mustard powder samples was observed. Therefore, the lowest and the highest reduction rates belonged to hot water (90.22%-91.66%) and steam (91.01%-92.15%) heat processes during (5-20 min). It has been previously shown that at the same temperature, wet (steam) heating methods had more severe effect on the reduction of total phenolic compounds as compared to dry (hot air) methods (Ghaderi Ghahfarokhi *et al.,* 2011). But the interesting result of our research is between two different wet heating media (steam and hot water), hot water might act substantially milder than the other and even dry (hot air) heating medium. This result is positively coordinated with the reduction of myrosinase activity; therefore more phenolic contents might be saved in the samples with higher residual enzyme activity. It might be concluded that in blanching process, the circulation rate of the heating medium that affect its heat transfer coefficient should be adjusted to provide optimum enzymatic activity as well as the total phenolic contents.

- *The effect of different heating processes on the phenolic compounds*

Tables 6, 7 and 8 present the effect of different deheating processes on the phenolic compounds of white mustard powder.

Table 6. The effect of hot air heating process on the concentration (mg/kg) of different phenolic compounds

Phenolic component	Heating time (min)					
	0	5.00	8.75	12.50	16.25	20.00
Hydroxytyrosol	653.89± 24.37^{a}	535.64±9.33^{b}	317.04±16.14^{c}	211.50±10.54^{d}	89.92±9.67^{e}	19.09±2.23^{f}
Tyrosol	325.38±18.18^{a}	317.30±10.84^{a}	322.44±6.49^{a}	287.94 ±4.38^{b}	251.26±8.80 ^{b}	208.15±12.56^{c}
Sinapine	2505.0±117.50^{d}	2606.71±50.8^{d}	2741.13±59.60^{dc}	3008.4±34.40^{cb}	3228.49± 55.0^{ba}	3450.98±83.9^{a}
Caffeic acid	0±0.00^{c}	0±0.00^{c}	0±0.00^{c}	6.37±2.06^{c}	15.76± 3.06^{b}	51.39±4.36 ^{a}
Syringic acid	126.51±3.94^{a}	121.46 ±5.95^{a}	67.86±5.33^{b}	23.95±1.62^{c}	0±0.00^{d}	0±0.00^{d}
Sinapic acid derivative	316.66±5.83^{a}	306.88±7.84^{a}	268.66±7.32^{b}	244.65± 9.38^{b}	207.17±8.00^{c}	187.45±9.07 ^{c}
Flavanomarein	34.325±3.79^{a}	35.87± 4.33^{a}	32.45±3.16^{ab}	30.47±3.98^{abc}	20.27±3.09 ^{bc}	18.40±2.43 ^{c}
Sinapic acid	66.270 ±2.36^{a}	61.91±2.27 ^{ab}	58.91± 3.50^{ab}	57.75±3.34 ^{ab}	52.62±3.12^{b}	50.63 ±4.60^{b}

Values in same row with same superscript are not significantly (P<0.05) different.

Table 7. The effect of steam heating process on the concentration (mg/kg) of different phenolic compounds

Phenolic component	Heating time (min)					
	0	5.00	8.75	12.50	16.25	20.00
Hydroxytyrosol	653.89±24.37^{a}	515.11b±3.85^{b}	314.07±6.85^{c}	194.91±9.22^{d}	87.98± 9.83^{e}	10.85±2.19^{f}
Tyrosol	325.38 ±18.18^{a}	302.66±15.80 ^{a}	308.94±4.89 ^{a}	285.17±7.41^{ab}	239.46±11.95 ^{bc}	195.42±11.87 ^{c}
Sinapine	2505.0±117.50^{c}	2496.34±7.0 ^{0c}	2591.90±112.10^{c}	2869.84 ±181.8^{bc}	3015.19 ±47.70^{ab}	3314.10± 17.8^{a}
Caffeic acid	0±0.00 ^{c}	0±0.00 ^{c}	3.03±0.28 ^{c}	6.04±0.10 ^{c}	25.74±4.47 ^{b}	49.74±3.34 ^{a}
Syringic acid	126.51±3.94 ^{a}	105.43±7.06 ^{b}	63.58±7.71 ^{b}	9.11±3.84^{d}	0.51 ±0.73^{d}	0±0.00^{d}
Sinapic acid derivative	316.66±5.83^{a}	304.35 ±5.81^{ab}	277.55±6.19 ^{b}	219.13± 9.44^{c}	198.41±12.72$^{c\ d}$	166.74±6.59 ^{d}
Flavanomarein	34.325±3.79^{a}	38.66 ±2.05^{ab}	26.01±1.58 ^{bc}	22.56±3.76 ^{cd}	17.38 ±0.57^{cd}	14.16±0.74 ^{d}
Sinapic acid	66.270 ±2.36^{a}	63.08±3.84 ^{a}	60.59±2.37 ^{ab}	51.05±1.52 ^{bc}	48.52±3.82 ^{c}	43.42±2.12 ^{c}

Values in same row with same superscript are not significantly (P<0.05) different.

Table 8. The effect of hot water heating process on the concentration (mg/kg) of different phenolic compounds

Phenolic component	Heating time (min)					
	0	5.00	8.75	12.50	16.25	20.00
Hydroxytyrosol	653.89±24.37 ^{a}	626.56±9.11 ^{a}	518.46±15.67^{b}	421.59±15.86^{c}	280.04±15.00^{d}	115.50±11.32^{e}
Tyrosol	325.38± 18.18 ^{a}	335.43±10.47 ^{ab}	324.16± 7.40^{ab}	308.28± 6.43^{ab}	277.21 ±17.09^{bc}	249.32±14.73^{c}
Sinapine	2505.0± 117.50^{b}	2562.00±11.70^{b}	2535.8±8.00^{b}	2621.78±14.90 ^{b}	2649.84±68.70^{b}	2889.83±14.8^{a}
Caffeic acid	0±0.00^{c}	0±0.00^{c}	0±0.00^{c}	1.74± 0.56^{c}	12.04±2.39^{b}	19.96 ±2.16^{a}
syringic acid	126.51±3.94 ^{a}	126.29±5.59 ^{a}	110.39± ^{a}	89.87±2.91 ^{b}	71.74±3.27 ^{c}	63.07±2.47 ^{c}
sinapic acid derivative	316.66±5.83^{a}	321.81±5.54 ^{a}	314.65±6.05 ^{a}	295.34±8.54^{ab}	275.85±6.31 ^{b}	245.19± 7.46^{c}
Flavanomarein	34.325±3.79^{a}	39.88±2.61 ^{ab}	37.49±0.75 ^{ab}	33.32±1.11$^{a\ b}$	28.47±1.65^{bc}	20.38±3.40 ^{c}
sinapic acid	66.270± 2.36 ^{a}	67.22± 1.68^{ab}	66.93± 1.41^{ab}	60.60±0.97 ^{abc}	64.16±1.47^{bc}	58.26±1.61^{c}

Values in same row with same superscript are not significantly (P<0.05) different.

As shown in the results sinapine and hydroxytyrosol are the major phenolic compounds present in the mustard powder at the levels of 2505.02 mg/kg and 653.89 mg/kg, respectively. During heating processes different variations in phenolic compounds has been observed. Hydroxytyrosol was decreased by approximately 82%, 97% and 98% after heating for 20 minutes by hot water, steam and hot air, respectively. Hydroxytyrosol and tyrosol showed high heat sensitivity that might be related to the simple structure and low molecular weights of these phenolic compounds (Baldioli *et al.,* 1996).

On the other hand Flavanomarein with high molecular weight (450.36 g·mol−1) and complex structure (Cerretani *et al.,* 2009) showed a good heat stability and its concentration did not change significantly during different deheating processes. Sinapine (colin ester of sinapic acid) is one of the phenolic acid that its concentration in mustard powder sample was interestingly increased during processing by all of the heating media (Tables 6, 7 and 8). It might be emphasized that hot air and hot water showed the most and the least severe effect by approximately 37% and 15% increase in sinapine concentration, respectively.

There are also some newly formed phenolic compounds. Caffeic acid [(3,4-Dihydroxy-phenyl) -2-propenoic acid] was not existed in the original mustard powder but formed after heat treatments and its concentration significantly increased with time. Therefore after 12 min of hot water and air heating its level reached 1.74 mg/kg, 6.37 mg/kg and steam has similar effect in shorter time (8 min). Caffeic acid is an organic compound, classified as hydroxycinnamic acid and is one of the active compounds exhibiting biological activities. Numerous studies investigated the pharmacological properties of caffeic acid including inhibition on cancer cell proliferation (Chuu *et al.,* 2012), powerful antioxidant (Chen *et al.,* 2001), immunomodulatory (Park *et al.,* 2004), antimicrobial (Stojkovic *et al.,* 2013), anti-aging (Jeong, *et al.,* 2011), and anti-inflammatory (LeBlanc *et al.,* 2012) and antidiabetic activity (Dhungyal *et al.,* 2014). It might be concluded that heating by steam and hot air might positively have effect on mustard powder by forming a nutritional valuable phenolic compound. Ee *et al.* (2011) investigated the effect of roasting on the antioxidant activity of different types of seeds and reported that oxidative stability of the roasted seeds might be increased because of the release of phenolic compounds from the partially destroyed cell structure or progressing maillard reactions and formation of some type of compounds with antioxidant activity. Vujasinovi *et al*. (2012) confirmed

that the chemical composition of seeds might be changed during roasting and might result in increasing the oxidative stability. Shrestha *et al.* (2013) reported that heat stability of mustard might be increased during thermal processes due to formation of new phenolic compound, however, the chemical mechanisms of this formation have not been yet clearly evaluated and remain ambiguous.

- *Process Optimization by Response Surface Method (RSM)*

Response surface methodology was applied to determine the optimum conditions for deheating powdered yellow mustard seed to produce a product with the best antimicrobial activity, color and total phenolic contents. According to the statistical analysis 5min of hot air heating in the autoclave might be introduced as the optimum process as shown in Table 9**.**

Table 9. Functional properties of the optimal deheated mustard powder

Properties	Expected results
MIC against	
Bacillus subtilis	-*
Bacillus coagulans	-
Saccharomyces cerevisiae	1.6
Zygosaccharomyces rouxi	3.1
Color Characteristics	
a*	2.09
b*	34.50
L*	67.94
Residual enzyme activity (µg glucose released / min)	643.37
Total Phenolic contents (mg/kg)	5376.42

*without inhibitory effect (MIC>3.5%)

Conclusion

Hot/pungent flavor of mustard has usually limited its usage in the food industry. In this study, different combinations of the heating time and methods were applied to partially inactivate myrosinase and save antimicrobial activity simultaneously. One of the main factors that significantly influenced the residual enzymatic activity was the moisture content of blanched product that seems to be a limiting parameter for hot water blanching of the relatively dried products such as plant seeds. According to the statistical analysis of the results, five minutes of hot air heating might be introduced as the optimum blanching process to produce a deheated yellow mustard powder with approximately 31% lesser enzyme activity (less hot flavor) and also suitable color characteristics and antimicrobial function and the least change in phenolic compounds. The effects of this multifunctional additive, produced under optimal conditions, on physicochemical and microbiological properties of foodstuffs such as different kinds of sauces need further studies.

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