Effect of various process treatment conditions on the allyl isothiocyanate extraction rate from mustard meal

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Abstract Allyl isothiocyanate (AITC), which has the potential to be used as flavoring, antibacterial, antifungal, antifermentative and antibrowning agent in food industry, was extracted from the pretreated MM by distillation technique. The mustard meal was analyzed for the proximate composition and the metals Fe, Mg and Zn. At the optimum pretreatment conditions of temperature 60 °C, time 120 min and pH 4.5, the effect of fractional distillation, mesh size and different additives was studied. Considerable effect of mesh size was observed, as the mesh size was decreased from 1,690 to 400 µm, the allyl isothiocyanate content was increased from 99.15 to 337.11 mg/100 ml. Addition of magnesium chloride (0.05 g/l to 0.2 g/l) and L-ascorbic acid (1 g/l to 5 g/l) increased allyl isothiocyanate from 257.79 to 317.28 mg/100 ml and 316.77 to 396.60 mg/100 ml respectively whereas the addition of the magnesium chloride and L-ascorbic acid in combination did not affect the AITC extraction rate as compare to their addition in single effect.

Keywords Mustard meal · Allyl isothiocyanate · Hydrolysis · Mesh size

Introduction

India grows five different rapeseed-mustard species, among these *Brassica juncea* is the most important, covering more than 75% of the total area followed by *B. campestris* and *Eruca sativa*. Antinutritional factors like phytic acid, glucosinolates and trypsin inhibitor have been reported in mustard seed meal and mustard protein isolates. The nutritional quality of the rapeseed protein is high and its protein value is comparable with soybean and the FAO/WHO reference protein (Jones 1979). But, due to high levels of toxic compounds like glucosinolates, phytic acid and fibre content in the hulled mustard meal (MM), it cannot be used in human diets (Serraino and Thompson 1984).

A procedure is described for the removal of glucosinolate from whole mustard or rapeseeds, or in the cake obtained from these seeds, by enzymic conversion to isothiocyanate, followed by steam distillation (Ahuja and Sherry 1979; Deven et al. 2000). Effect of pretreatment conditions on the allyl isothiocyanate (AITC) from mustard meal was studied. The pretreatment conditions are the important parameters which can affect the amount of AITC in the distillate (Garg 2006). The volatile oil, allyl isothiocyanate (AITC), which makes the mustard seed unpalatable, is usually synthetically produced. The extraction of the volatile oil is based on the fact that the mustard seed contains thioglucosides (sinigrin in black mustard; sinalbin in white mustard). Sinigrin may be hydrolyzed by the enzyme myrosinase, which is a thioglucosidase and contained in the seeds themselves. The products of this hydrolysis are glucose, hydrogen sulphate, and AITC. The last-named product is a highly valuable volatile oil (specific gravity 1.020) and may be recovered by distillation

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(Szakacs-Dobozi et al. 1988). This substance has a number of industrial, pharmaceutical and agricultural uses. The potential use of AITC as antibrowning, antibacterial and fungistatic agent has been reported (Ohta and Kawakishi 1998; Kanemaru and Miyamoto 1990). AITC is also being permitted for use as an anti-fermentative agent in wine-making (Scaroni et al. 1993).

Ludikhuyze et al. (2000) investigated the potential of several intrinsic (MgCl_2 , ascorbic acid, pH) and extrinsic (temperature, pressure) factors for controlling/altering activity of myrosinase from broccoli against the glucosinolate and sinigrin. A combination of MgCl_2 and ascorbic acid was found to enhance enzymic activity. Mustard seeds do not yield enough volatile oil upon the decomposition of sinigrin to make its recovery worthwhile. To obtain the desired result, it is essential that the sinigrin is decomposed by hydrolysis and this can be accomplished by using the enzyme contained in the seeds themselves (Szakacs-Dobozi et al. 1988). To have a maximum recovery of AITC it is necessary to decompose maximum amount of glucosinolates prior to distillation and this may be achieved by giving the proper pretreatments conditions. The pretreatment conditions mainly time, temperature and pH were optimized by using Response surface methodology (Garg 2006). The recovery of AITC may be affected by the composition of the mustard meal and various other factors. Therefore, in order to have maximum extraction of AITC from mustard meal, the effect of mesh size and different additives was observed in the present study.

Materials and methods

Materials

Mustard seeds (*Brassica juncea*) variety was procured from the local market Sangrur, Punjab. Seeds were cleaned, dried and stored in airtight container for further use. Chemicals used under the study were of analytical grade.

Preparation of mustard meal

Mustard meal (MM) was prepared by extracting the oil from the mustard seed (*Brassica juncea*) with the help of screw expeller at Sangrur, Punjab. Five kilogram of mustard seeds were used for the preparation of mustard meal. Expellers consist of a rotating screw shaft in cylindrical barrel. The barrel has flat steel bars mounted edgewise around the inside and spaced to allow oil to flow from the bars while retaining the solid material with in. The solid material retained in barrel is discharged from the barrel end. This mustard meal was ground in to powder and packed in a container for further use. This ground mustard

meal was further defatted by hexane and the residual solvent left was evaporated. This mustard meal was ground in to powder and packed in a container for further use.

Methods

Pretreatment of mustard meal

The ground and sieved MM prepared from the mustard seed was taken for the enzymatic hydrolysis. The meal (5 g) was incubated in a round bottom flask, at the optimum temperature, time and pH combination as reported by (Sharma et al. 2008), consisting of 250 ml water and 25 ml of ethyl alcohol. The pH of mixture was maintained by using L- ascorbic acid. The mixture was distilled in steam and 150 ml of the distillate was collected in a 200-ml measuring flask containing exactly 25 ml of silver nitrate solution and 10 ml of ammonium hydroxide solution.

Effect of mesh size

Ground mustard meal was sieved through the different mesh size of 1,680, 1,000, 707, 595, 500 and 400 μm to study the effect on the extraction of the AITC at the selected optimum pretreatment conditions of time, temperature and pH i.e. 120 min, 60 °C and 4.5 respectively.

Effect of MgCl_2 and ascorbic acid

The worked out mesh size from the study was treated with the MgCl_2 ; at the level of 0.05, 0.1, 0.15, 0.2 and 0.25 g/l and L-ascorbic acid at the level of 1, 2, 3, 4 and 5 g/l; separately to find out the effect on the extraction of the AITC. The optimum concentrations of both the additives were also tried in combination.

Physico-chemical analysis of mustard meal

Mustard meal was subjected to moisture, protein, crude fibre, crude fat and ash content by using standard methods (Ranganna 1986).

Determination of minerals

The organic material was removed by both dry ashing and wet digestion method as per the standard procedure (Ranganna 1986). These digested samples were then analyzed for the determination of iron, magnesium and zinc by atomic absorption spectrophotometer (AAS). The standard curves for the iron, magnesium and zinc are given in Figs. 1, 2 and 3 respectively.

The data were regressed and the models were developed to evaluate the mineral content directly. The Eqs. 1, 2 and 3

Fig. 1 Effect of mesh size (micron) on the AITC content (mg/100 ml)

represent the regression equations for the estimating iron, magnesium and zinc respectively. The absorbances were noted at the wavelength (λ) of 248.3, 285.2 and 213.9 nm for iron, magnesium and zinc respectively.

$$y = 0.4044x - 0.0027(\text{Iron}) \quad (1)$$

$$y = 0.7601x - 0.0158(\text{Magnesium}) \quad (2)$$

$$y = 0.0482x - 0.0009(\text{Zinc}) \quad (3)$$

Where y = absorbance and x = concentration of mineral.

Determination of the AITC

The amount of AITC formed from the MM was determined as per the method adopted by Sharma et al. (2008). The allyl isothiocyanate was distilled into a known excess of silver nitrate solution, and the excess of silver nitrate solution is determined by titration with standard ammonium thiocyanate solution.

Statistical analysis

The data thus obtained regarding variations in the compositions were analyzed for significant changes by student's

Fig. 2 Effect of magnesium chloride concentration (g/l) on the AITC content (mg/100 ml)

Fig. 3 Effect of ascorbic acid concentration (g/l) on the AITC content (mg/100 ml)

t -test (Mendenhall and Sinsich 1987). Fisher least significant difference (LSD) procedure was applied to the difference in the experimental data (Montgomery 2001).

Results and discussions

Allyl isothiocyanate (AITC), which has the potential to be used as flavoring, antibacterial, antifungal, anti fermentative, and antibrowning agent in food industry, was extracted from the pretreated mustard meal by distillation. The mustard meal was prepared from the mustard seed with the help of conventional expeller. Table 1 shows the proximate composition of mustard seed and meal used for the further study. Mustard meal contained higher amount of moisture content, crude protein, ash and crude fibre than whole seed. The increase may be attributed to the removal of the oil and the higher water absorption capacity of mustard meal. The results are in agreement with the findings of (Talati et al. 2004).

Minerals in mustard meal

The mineral composition, determined using Atomic Absorption Spectrophotometer (AAS), is shown in the Table 2. The composition of iron, magnesium and zinc was found to be 329.92, 1.75 and 36.22 mg/l respectively by dry ashing method whereas these values were observed as 363.3, 1.63 and 41.06 mg/l by the wet digestion method. The observed values were analyzed by t -test for equality of the means of two independent lots. No

Table 1 Proximate compositions of mustard seed and meal

Parameters	Seed	Meal
Moisture, %	6.1±0.21	9.1±0.42
Oil, %	33.6±1.1	11.4±0.72
Crude protein, %	24.3±1.9	39.1±1.5
Crude fiber, %	6.9±0.92	11.8±0.86
Ash, %	4.1±0.49	6.5±0.37

Table 2 Composition of various metals in the mustard meal

Metal	Dry ashing (mg/l)	Wet digestion (mg/l)	t value
Iron ^a	329.9±1.2	363.3±1.1	2.94
Magnesium ^a	1.7±0.02	1.6±0.032	1.51
Zinc ^a	36.2±0.74	41.0±0.86	3.56

^a Results are average of three individual experiments

significant difference was found in the composition of the minerals ($P<0.01$) obtained from the dry ash and wet digestion method. Dominances of iron and zinc in MM over magnesium were in accordance with the study carried out on metal content of mustard meal accordingly mustard meal was reported to contain 212.5 mg/l iron, 53.5 mg/l zinc and 1.3 mg/l magnesium (Bell 1989). The variation in the mineral content may be due to the variety, soil and the climatic conditions under which the seeds were grown.

Effect of mesh size on the AITC content

The influence of mesh size on% AITC is presented in Fig. 1. The data indicated that as the mesh size was decreased from 1,690 to 400 μm , the allyl isothiocyanate content increased from 99.15 to 337.11 mg/100 ml. From the Fig. 1, it is evident that mesh size is inversely affecting the allyl isothiocyanate extraction rate. Increased rate of AITC content was due to the increase in the exposed surface area for the enhancement of the reaction. In addition, reduction in mesh size may result into more fine particles which probably led to more efficient glucosinolate transformation. In a previous study (Sethi and Anand 1975) the volatile oil (allyl isothiocyanate) was found varying from 0.08 to 0.8% in the nine developed strains of *Brassica juncea*. From the Figure, it is evident that the extraction rate of AITC was sharply increased up to the mesh size of 707 μm thereafter the extraction rate was slower comparatively. Therefore, the mesh size of 707 μm was undertaken to check the effect of additives.

Effect of magnesium chloride and ascorbic acid on the AITC content

The effect of magnesium chloride on the AITC extraction rate is shown in Fig. 2. Results suggest that as the concentration of magnesium chloride increased from 0.05 g/l to 0.2 g/l, the amount of allyl isothiocyanate was increased from 257.79 to 317.28 mg/100 ml. No increase in the AITC content was observed with the further increase in concentration upto 0.25 g/l (Fig. 2). The least significant difference (LSD) was calculated as 32.84 for the experimental data in the given range (Table 3). The data indicated that the effect of magnesium chloride on the AITC extraction rate was significant when the magnesium chloride used was greater than the 0.2 g/l. Lesser than 0.2 g/l, LSD value were found to be greater than the observed difference between each pair of averages indicating that the effect was non-significant.

From the increase of L-ascorbic acid concentration, 1 to 5 g/l, the allyl isothiocyanate content was increased from 316.77 to 396.60 mg/100 ml (Fig. 3). Further increase in the concentration of L-ascorbic acid upto 6 g/l, similar pattern with respect to allyl isothiocyanate content was observed as for the magnesium chloride. The least significant difference (LSD) value was obtained as 43.88. The observed difference between 1–3, 1–4 and 1–5 g/l value was greater than the LSD value of 43.88 whereas the difference between 1 and 2 g/l value was lesser, which indicated the significant effect of L-ascorbic acid at the concentration of 3 g/l or greater than 3 g/l (Table 3).

The enhancement in AITC in presence of L-ascorbic acid and magnesium chloride may be due to catalyzing the hydrolysis of the glucosinolates. The findings are in accordance with the Ludikhuyze et al. (2000) wherein the study reported that L-ascorbic acid and magnesium chloride enhances the conversion of glucosinolate to AITC from broccoli.

The combined effect of magnesium chloride and L-ascorbic acid was studied at a level of 0.2 g/l and 4 g/l respectively where the maximum amount of AITC

Table 3 Analysis of variance and LSD value for the additives (magnesium chloride and ascorbic acid) data

Source of variation	Sum of squares	Degrees of freedom	Mean square	LSD _(0.025,16) value
Magnesium chloride				
Between groups	309804.70	3	103268.2	32.84
Within groups	9483.92	16	592.74	
Total	319288.62	19		
Ascorbic acid				
Between groups	488765	3	162921.7	43.88
Within groups	17133.35	16	1070.834	
Total	505898.3	19		

LSD least significant difference

content was obtained. When added in combination, the AITC was found to be 396.60 mg/100 ml indicating that there was no enhancement in the yield of AITC as compare to the addition of these additives in their single effect.

Conclusion

As the mesh size was decreased, the allyl isothiocyanate content was increased from 99.15 to 337.11 mg/100 ml. Addition of magnesium chloride and L-ascorbic acid increased allyl isothiocyanate from 257.79 to 317.28 mg/100 ml and 316.77 to 396.60 mg/100 ml respectively where as the addition of the additives in combination did not affect the % AITC extraction rate.

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