CORVINUS UNIVERSITY of BUDAPEST

Faculty of Food Science

PhD thesis

Development of radio frequency heat treatment technology for the production of non-pungent mustard (*Sinapis alba* L.) seed flour

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1. INTRODUCTION

Due to its characteristic flavour and medicinal effect, white mustard (*Sinapis alba* L.) became a renowned herb and medicinal plant in ancient times. With its original home in the basin of the Mediterranean Sea, it was already known all over Europe back in the XII. century, arriving in Hungary through the Germanic territories. In folk medicine, ground mustard seed is applied as a poultice. Mustard plaster offers quick relief primarily for acute pain, vertebral disk problems and neurotises. As a home remedy, it is used for curing bile and liver diseases, constipation, rashes and also for preventing arteriosclerosis.

Similarly to oil seeds used in the food industry, in nutrition science mustard seed with its high protein and oil content is suitable for the production of excellent foodstuff, additives and feeds.

In Hungary mustard seed is mainly used as a condiment; however, its application is highly justified in both human and animal nutrition, as it is a good source of a protein with its relatively high essential amino acid content, ω-3, ω-6 fatty acids and biologically active compounds. As an additive, it may feature favourable colloid-chemical properties (emulsifying properties, water- and fat binding capacity) in products.

Like other oil seeds, mustard seed is also rich in phenolic compounds which, due to their high antioxidant and free radical scavenging capacities, are considered protective agents against cardiovascular and certain tumorous diseases. Moreover, they are cholesterol and gluten-free, so the consumption of mustard seed may have health promoting effect.

The cultivation of mustard is successful in temperate zones and especially economical in Hungary. The use of mustard seed for food industrial and feeding purposes requires a sound knowledge of the chemical composition of mustard varieties in commercial production and also their comparison. In spite of its beneficial chemical composition, the wide scale use of mustard seed is greatly hindered by its pungent flavour and the high level of erucic acid in its oil. The high erucic acid content can be reduced by breeding; a variety of this kind is already used in commercial production. The pungent flavour of mustard is caused by breakdown products of glucosinolate hydrolysis induced by the myrosinase enzyme; therefore the inactivation of the myrosinase enzyme removes this unfavourable property. As heat treatment inactivates myrosinase, the gentle, energy–saving and environmentally sound radio frequency heat treatment was applied for the production of non-pungent mustard flour.

2. OBJECTIVES OF THE RESEARCH

The aim of the study was to produce enzyme inactivated mustard seed flour, which is a favourable source of the nutritional components and it has advantageous techno-functional properties. Production of high quality food preparations has two equal conditions: choosing the proper raw material and applying minimal processing technology, which has no harmful effect on the advantageous properties of the raw material, therefore the following objects were proposed:

- Determination of the nutritional compounds (protein content and amino acid composition, oil content and fatty acid composition, quantity of phenolic compounds and free radical scavenging activity, as well as myrosinase enzyme activity and glucosinolate content) and physico-chemical properties (water- and fat binding capacity, emulsifying properties) of the mustard seed to be treated previous to the radio frequency heat treatment.

- Determination of the radio frequency heat treatment parameters (temperature and moisture content) which are suitable for inactivation of myrosinase enzyme, and has no harmful effect on protein content and biologically active compounds.

- In addition to inactivation of myrosinase enzyme, heat treatment can cause changes in the nutritional properties; therefore further aim was the examination of nutritional properties and colloid-chemical properties after the radio frequency heat treatment, as well as characterisation of changes caused by the treatment.

- Analysing the utilization possibilities of the enzyme inactivated mustard seed in the food industry with special emphasis on meat industry applications.

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3. MATERIALS AND METHODS

In the course of the study chemical composition, myrosinase enzyme activity, biological active compounds and techno-functional properties of different mustard seed varieties originating from different growing places were examined between 2002 and 2005.

3.1. Materials

Examined mustard varieties (*Sinapis alba* L.):

Budakalászi sárga (Date of Listing: 1972)
Tilney (Date of Listing: 1999)
Veronika (Date of Listing: 2004)
Zlata (variety included in the National list of varieties of Czech Republic)
Viscount (variety included in the National list of varieties of Great Britain)
Low erucic acid content varieties: LM-1 (Marci, Date of Listing: 2005)
LM-2

Mustard varieties were provided by Károly Róbert College and Monortrade Ltd.

Growing places:

Growing place of Monortrade Ltd.,
Budakalász,
Kiskunlacháza,
Kaposvár,
Szombathely,
Eszterág,
Putnok,
Gyöngyös.

Utilization possibilities of mustard seed flour were investigated in the case of „Olasz", „Zala" and liver meat products in laboratory conditions.

Experimental work was supported by NKFP project No. 4/0005/2002.

3.2. Methods

Applied sample treatment methods

Radio frequency heat treatment:

Mustard seed was radio frequency heat treated in the operator space of Brown-Boveri generator (13.5 MHz) in horizontal workspace.

Sample preparation:

Prior to the experiments mustard seed was ground by coffee grinder. Ground mustard seed was used for determination of moisture content, protein content and amino acid composition. For further examinations ground mustard seed was defatted by petroleum ether (40-70 °C) in Soxhlet apparatus and dried. Defatted mustard seed was ground to 200 μm particle size.

Determination of chemical composition

Protein content of mustard seed:

The protein content of whole mustard seeds was determined by Kjeldahl method (N x 6.25) using automatic Kjel-Foss apparatus.

Amino acid composition of mustard protein:

The amino acids were separated by ion exchange chromatography in Biotronik LC 3000 amino acid analyser after hydrolysis of ground, fat containing mustard seed.

Oil content of mustard seed:

Oil content of ground mustard seed was extracted in Soxhlet apparatus using petroleum ether (40-70 °C) (MSZ EN ISO 734-1:2000), then the solvent was evaporated. Quantity of the extracted oil was determined gravimetrically.

Fatty acid composition of mustard oil:

Mustard oil extracted by petroleum ether was saponificated and fatty acids were separated by gas chromatographic method (MSZ 19928-79).

Tocopherol content:

Sample to be treated was saponificated, tocopherol compounds were separated by HPLC method (SPEEK et al., 1985).

Total phenolic content:

Methanolic extract was prepared from defatted mustard seed flour, total phenolic content was measured by Folin-Ciocalteu reagent, and calculated on the basis of gallic acid calibration curve.

Free radical scavenging activity:

Radical scavenging activity of methanolic extract prepared from defatted mustard seed was measured on DPPH radical and expressed as Trolox equivalent (YAMAGUCHI et al., 1998).

Total glucosinolate content:

Total glucosinolate content was determined on the basis of quantity of glucose liberated by hydrolysis of glucosinolate compounds according to VANETTEN et al. (1974). Quantity of glucose liberated by the added myrosinase was measured by spectrophotometrical method using GOD–PERID glucose assay kit.

Myrosinase enzyme activity:

Myrosinase enzyme activity of mustard seed was determined on the basis of quantity of glucose liberated through the action of endogenous myrosinase enzyme from intact glucosinolate compounds (VANETTEN et al., 1974). Quantity of glucose liberated by endogenous myrosinase was measured by spectrophotometrical method using GOD–PERID glucose assay kit.

Heat sensitivity of myrosinase enzyme:

Crude myrosinase enzyme was prepared from petroleum ether defatted mustard seed flour by method according to OWUSU-ANSAH and MARIANCHUK (1991).

Solution of prepared myrosinase enzyme was added to buffer solution heated to the proper temperature and was heat treated for proper period. Heat treated enzyme solution was added to ice cool buffer, and the prepared sample was stored in refrigerator until the determination of enzyme activity. The enzyme activity before and after heat treatment was determined on natural glucosinolate compounds using 100% inactivated mustard seed flour as substrate. Quantity of glucose liberated by the action of myrosinase enzyme was determined by GOD-PERID glucose oxidase–peroxidase reagent after filtration and deproteinization of the reaction mixture. (VANETTEN, et al. 1974).

Pungency threshold of radio frequency heat treated mustard seed:

Sensory analysis of pungency was carried out with mustard seed having known myrosinase activity. 1.5% suspensions were prepared from ground mustard seed with tap water. In the course of the sensory analysis 3 assessors evaluated pungency of the suspensions.

Isothiocyanate content:

Quantity of isothiocyanate compounds liberated through the action of added myrosinase enzyme was measured by spectrophotometrical method and calculated on the basis of potassium thiocyanate calibration curve. (JOSEFFSON, 1968).

Colloid chemical properties

Emulsifying properties (emulsifying activity and emulsion stability):

Emulsifying activity and emulsion stability of mustard seed flour was determined by method according to YASUMATSU et al. (1972).

Water- and fat binding capacity:

Water- and fat binding capacity of mustard seed flour was determined by method according to LIN et al. (1974).

Protein solubility:

Defatted mustard seed was suspended in distilled water, solubility of mustard protein was determined in pH range of 5–7. The prepared suspension was centrifuged and filtered, the protein content of the filtrate was determined by Lowry method with Folin-Ciocalteu reagent. Quantity of soluble protein was determined according to bovine serum albumin calibration curve.

Gel forming properties:

Suspension prepared from mustard seed flour was homogenized. The homogenized suspension was diluted to various concentrations, heat treated and cooled. The firmness of the formed gel was examined by reversing the test tube. The concentration was registered when gel flow was not observed. The firmness of the formed gel was also examined visually.

Examination of meat products prepared with mustard seed flour

Texture properties of products made with mustard seed flour:

The textural testing of the meat products was completed on a LLOYLD LR5K plus texture analyser and NEXYGEN 4.1. software was used to operate the instrument and calculate the results.

Sensory analysis of meat products made with mustard seed flour:

Ten assessors participated in the sensory analysis of the meat products made with mustard seed flour. The results of the complete sensory profile analysis were evaluated by ProfiSens software, which was developed in the Sensory Laboratory of Corvinus University.

3.3. Statistical methods applied for evaluation of results

In the course of evaluation of the results correlation calculation, t-test, analysis of variance and Tukey's pairwise comparison as well as canonical discriminant analysis were used.

4. RESULTS

The effect of growing location, vintage, and variety on the chemical composition and techno−functional properties of mustard seed was evaluated by an analysis of variance (α=0.05). According to the results protein content, oil content, linoleic acid content of mustard oil, total polyphenol content and free radical scavenging activity of mustard seed are vintage dependent properties. The effect of growing location on protein content, glucosinolate content, myrosinase enzyme activity, total polyphenol content, as well as water- and fat binding capacity is verified statistically. Stability of emulsion prepared of ground mustard seed as well as oleic acid, erucic acid, linoleic acid and linolenic acid content of mustard oil is affected significantly by variety.

On the basis of the results a negative, significant (α=0.001) correlation was established between the oil content and protein content of the examined mustard varieties.

There is a linear relationship between the free radical scavenging activity and total phenolic content, the positive correlation is significant (α=0.001).

Fatty acid composition is a variety dependent property. As a consequence of the erucic acid decrease, the oleic acid content was increased. According to the results, the negative correlation between the erucic acid and oleic acid content is significant (α=0.001). In the case of the low erucic acid variety, the linoleic acid content was 3-4% higher than the measured values of the other examined varieties.

According to the results of the canonical discriminant analysis, composition of the mustard seed is influenced in the highest degree by the variety. The effect of the year was the highest in the case of oil content and free radical scavenging activity. The group configuration was determinated by the fatty acid composition of the mustard oil.

In the course of the preliminary heat treatment experiments, four different varieties (Budakalászi sárga, Tilney, Zlata and LM-1 low erucic acid content var., 2002) were heat treated under different parameters. Radio frequency heat treatments were carried out at temperature of 90 °C, 100 °C, 110 °C and 120 °C, the moisture content of the seeds was adjusted to 15%, and 18%. The effect of the heat treatment was measured by the decrease of myrosinase enzyme activity. Effects of the heat treatment on the nutritional compounds were also investigated.

On the basis of the preliminary heat treatment results, heat sensitivity of myrosinase enzymes originating from the four examined mustard variety is different, therefore the mustard variety was chosen, which contains the least heat sensitive myrosinase enzyme. According to the results, the LM-1 low erucic acid content variety contains the least heat sensitive enzyme. The inactivation of the enzyme can be described by a first order kinetic equation.

According to the preliminary experiments, the inactivation of the myrosinase enzyme is higher in the case of heat treatment carried out at the same temperature with higher moisture content.

In the course of establishing the optimum parameters of the radio frequency heat treatment the experiments were carried out with LM-1 low erucic acid content var. at 112 °C and 114 °C temperature, the moisture content of the seeds was adjusted to 14%, 16% and 18%. According to the results, 112 °C temperature and 16% moisture content were established as the optimum parameters of the radio frequency heat treatment for inactivation the myrosinase enzyme in mustard seed.

On the basis of the results obtained after heat treatment carried out at 112 °C temperature and 16% moisture content less than 20 U/g myrosinase enzyme activity could be measured, therefore it can be considered as verified that the heat treatment performed with the chosen parameters is effective.

There was no difference between the protein content of the RF treated and the untreated mustard seed. After RF treatment, increase of the mustard oil yield could be observed. Decrease of the glucosinolate content after radio frequency heat treatment varied between 10% and 30% depending on vintage and growing place. According to the results of treated and untreated mustard seeds, total polyphenol content and free radical scavenging activity increased by the effect of radio frequency heat treatment.

Radio frequency heat treatment carried out at 112 °C and 16% moisture content effected significant changes in certain cases of water- and fat binding capacity of ground mustard seed, and there was no change in the emulsifying activity. Stability of emulsions prepared from ground mustard seed is relatively low, but due to the radio frequency heat treatment the emulsion stability increased by 8%-132%.

Examining the emulsion stability of mixtures prepared from mustard seed and soy isolate in different ratios it can be established that stability of emulsion made of soy isolate can be improved by addition of enzyme-inactivated ground mustard seed, their application is the most advantageous in ratio of 1:1. According to the results, in the course of emulsion preparation, enzyme-inactivated mustard seed can be suitable for partial replace of import soy isolate.

In the course of the study of utilization possibilities, three different products were manufactured (Olasz, Zala and liver meat products) under laboratory conditions according to the original recipe and effect of the added myrosinase-inactivated mustard seed flour on rheological and sensory properties were evaluated. On the basis of firmness as well as ratio of gel and fat separation results there was no difference between the meat products containing myrosinase-inactivated mustard seed flour and the meat products manufactured according to the original recipe. According

to the statistical evaluation of the sensory analysis results, addition of myrosinase–inactivated mustard flour to the meat products has no significant effect on taste, odour, consistency and cutting surface (α=0.05).

5. New scientific results

1. On the basis of the results I established a negative, significant (α=0.001) correlation between the oil content and protein content of the examined mustard varieties.

2. I established that fatty acid composition of mustard seed is a variety dependent property. In the case of the low erucic acid variety, the linoleic acid content was 3-4% higher than the measured values of the other examined varieties.

3. On the basis of the preliminary heat treatment results I established that heat sensitivity of myrosinase enzymes originating from the four examined mustard variety is different, the LM-1 low erucic acid content variety contains the least heat sensitive enzyme. The inactivation of the enzyme can be described by a first order kinetic equation.

4. According to the results I established that 112 °C and 16% moisture content are suitable parameters of radio frequency heat treatment for production of non-pungent mustard seed flour. Radio frequency heat treatment carried with the properly adjusted parameters inactivates myrosinase enzyme, however, it has no harmful effect on nutritional compounds, water- and fat binding capacity as well as emulsifying properties of mustard seed flour. Stability of emulsion prepared from enzyme inactivated mustard seed flour was higher compared to the control.

5. I established that there is a linear relationship between the free radical scavenging activity and total phenolic content of mustard seed, the positive correlation is significant (α=0.001).

6. Examining the emulsion stability of mixtures prepared from mustard seed and soy isolate in different ratios I established that stability of emulsion made of soy isolate can be improved by addition of enzyme-inactivated ground mustard seed, their application is the most advantageous in ratio of 1:1. According to the results, in the course of emulsion preparation, enzyme–inactivated mustard seed can be suitable for partial replace of import soy isolate.

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Publications in journals

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