Effects of Legume Seed Flours on the Formation of Acrylamide and 5-Hydroxymethylfurfural (HMF) in a Model Reaction System

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Abstract: Seven legumes (black soybean, chickpea, green pea, lentil, mung bean, yellow pea, and soybean) were used to test their effect on the formation of acrylamide and HMF in an asparagines/glucose model reaction system. Five varieties inhibited the formation of acrylamide (from 10.3% to 55%) but all varieties improved the formation of HMF. Defatting legumes flour showed improved inhibition capacity for acrylamide formation, and a dose-effect relationship was observed for all the tested materials when 0.2, 0.4, and 0.6 g of the defatted flours were added. When the defatted-dehulled flours were added, acrylamide production was further inhibited in some treatments along with an increase in the HMF content. However, the addition of 0.2 g of hull flour from four varieties also decreased the acrylamide content and significantly increased browning. Defatted legume flours from five legumes eliminated the formed acrylamide, ranging from 28.5% of soybean to 5.9% of mung bean. Addition of the defatted-dehulled flours from four varieties also resulted in pH decrease and glucose depletion. Most of the 39 treatments studied in this research showed a negative relationship between the contents of acrylamide and HMF.

Key words: legumes flour; acrylamide; 5-hydroxymethylfurfural

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美拉德反应过程中 7 种豆粉对丙烯酰胺和羟甲基糠醛形成的影响

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摘要：丙烯酰胺和羟甲基糠醛是高温加工食品中产生的两种内源污染物。本文研究了 7 种豆类（黑豆、鹰嘴豆、绿豌豆、小扁豆、绿豆、黄豌豆和黄豆）的豆粉对天冬酰胺/葡萄糖反应体系中丙烯酰胺和羟甲基糠醛（HMF）形成的影响，以其为两者的控制提供参考。结果表明：采用全豆粉试验时，有 5 个品种的豆粉抑制丙烯酰胺（抑制率 10.3~55%）形成，但都促进 HMF 的产生；去皮脱脂后的豆粉更能抑制丙烯酰胺形成。有趣的是，其中 4 个品种的豆皮也能抑制丙烯酰胺的产生。进一步研究发现，有 5 个品种的脱脂豆粉能消除已形成的丙烯酰胺，消除率为 5.9%（绿豆粉）至 28.5%（大豆粉）。通过统计本研究的 39 种处理发现，丙烯酰胺与 HMF 的形成量呈现负相关。

关键词：豆粉；丙烯酰胺；羟甲基糠醛

Acrylamide and 5-hydroxymethylfurfural (HMF) are mainly formed through the Maillard reaction and can be regarded as the most important heat-induced contaminants in bread, bakery products, and other high temperature processed foods. Both of them are considered as potential carcinogens to humans or might be metabolized by humans to potentially carcinogenic compounds[1,2]. Many mitigation methods for acrylamide formation have been reported, including changes of recipes, formulations, and the processing technology, and removing its formation precursors[1,3]. However, no mitigation strategies specifically addressing the reduction of HMF content in foods are currently available[1].

Legumes are worldwide crops, which contain kinds of ingredients contributing to the reduction of acrylamide. The protein content in most legume seeds exceeds 20%, being of a rich source of lysine, up to 7% of the protein [4,5]. The ε-NH_2 of lysine was suggested to reduce acrylamide formation either by promoting competitive

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reactions and/or by covalently binding acrylamide that is formed in the system through Michael-type addition reactions [6]. Our previous research has indicated that the whole seed or the hull of soybean, pea, lentil, and chickpea contain high amount of phenolics[7], and some of the antioxidants have been reported to inhibit acrylamide formation[8]. The other compounds such as pectin, was also reported to inhibit the formation of acrylamide in fried potato chips by the formation of a barrier against oil[9]. Moreover, legumes are recommended for their excellent health-promoting benefits. Regular consumption of legumes food is suggested to reduce the risk of chronic disease, including obesity, diabetes, heart diseases, and cancers. In addition, legumes could be consumed in several forms, including whole-grain products, dehulled seed flour, and concentrated or isolated proteins. While, thermal treating would inactivate antinutritional factors before consumption [10].

All these chemical, nutritional, and processing characteristics suggest that the flour of the legume seed can be incorporated in bread, bakery products, and French crisps and that the flour may have a positive role in the inhibition of the formation of acrylamide, and possibly HMF. However, except for the findings from Vattem and Shetty[11], who found that chickpea batter could function as a thermal barrier that reduces formation of acrylamide in fried potato slices, reports on the effects of legume flour on the formation of acrylamide are not available.

In this research, the flours of whole grains, defatted grains, defatted-dehulled grains, and hulls from seven legume varieties were used to investigate their effects on formation of acrylamide and HMF, and the possible mechanism for their effects were also discussed.

1 MATERIALS AND METHODS

1.1 Materials

The dry legume seeds of two soybean varieties (*Glycine max* (L.)-yellow cultivar (cv. Prosy) and black cultivar (cv. C-1)-used in the current study were obtained from SB & B (Casseltor, North Dakota); mung bean (*Vigna radiata*) was obtained from the local market (produced in Taiwan); green pea (*Pisum sativum* L., cv. Stratus) was supplied by Meridian Seed LLC (West Fargo, ND); yellow peas (*P. sativum* L., cv. Golden) were supplied by Steve Marman Pulse (Bismark, ND); chickpea (*Cicer arietinum* L., cv. Amits) and lentil (*Lens culinaris* cv. CDC Richlea) were supplied by Agricare United (Ray, ND). Broken seeds, damaged seeds, and impurities were removed from the samples.

1.2 Reagents and chemicals

Asparagine, D-glucose, HMF, acrylamide standard (>99.9% purity), 3,5-dinitrosalicylic acid (DNS), glycerol, and sodium hydroxide were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA).

Soaking, blanching, and drying of the seeds. The legume seeds (approximately 1500 g) were washed using tap water and soaked in 5 mL water (*m/V*) at room temperature for 12 h. The soaked seeds were drained and then blanched in a stainless steel pail containing 20 kg of water at 82 ℃ for 5 min, with the temperature maintained constant using steam, provided by a boiler. The blanched legume seeds were immediately cooled in cold water, and the water was removed by placing the seeds on a stainless steel screen and vacuum dried at 65.1 ℃ for 48 h. At the end of the drying process, the moisture levels were 3~5%.

Preparation of seed samples. Different categories of legume samples, including whole-grain flour, defatted whole-grain flour, defatted-dehulled flour, and hull flour, were prepared. The hull was collected after blanching. The whole-grain (hull included) and hull flours were prepared using a ZM 100 Model mill (Retsch, USA) with a 0.5 mm sieve (for preparation of chickpea whole-grain flour, it was first ground by a plate mill before Z-milling). The flour was passed through a 60-mesh sieve. Subsequently, 20 g of whole-grain and dehulled legume flours were respectively defatted in 250 mL flasks with 5 mL hexane under constant stirring at 150 r/min using a magnetic stirrer (Lab-line Instruments Inc., Melrose Park, IL) at room temperature for 2 h. The residues obtained by filtration were defatted once more. After defatting, the residue was left under hood overnight for hexane to evaporate.

1.3 Effects of the flour from whole grains, defatted whole grains, defatted-dehulled grains, and hull on the formation of acrylamide and HMF

An equimolar asparagines-glucose model reaction system was used to test the effects of different parts of the legume seeds on the formation of acrylamide and HMF. Shortly, 30 mL stainless steel test tubes containing 4 mL asparagine (0.25 mmol/mL), 1 mL D-glucose (1 mmol/mL), and 0.2 g samples were capped and vortexed using a VM-3000 model mini vortexer. The mixture was incubated in an oil bath (4-L Oster professional style deep fryer, USA) at 160 ℃ for 15 min. The test tubes were removed and cooled using tap water, and the contents were decanted into a 14 mL centrifuge tube, washed using deionized water, made up to a final volume of 14 mL, and centrifuged using an Allegra 21R centrifuge (Beckman, USA) at 4000 r/min for 20 min. The process of browning, and the contents of acrylamide and HMF in the supernatant were determined by spectrophotometer and high-performance liquid chromatography (HPLC) respectively. For investigating the dose-effect relationship, the model reaction system was tested by the additions of 0.4 g and 0.6 g of the defatted whole-grain flour.

1.4 Effect of defatted legume flour on the elimination of acrylamide

The mixture in the test tubes comprising 1 mL acrylamide (100 μg/mL), 0.2 g defatted seed flour, and 4 mL deionized water was vortexed and allowed to react in an oil bath at 160 ℃ for 15 min. The residual acrylamide was detected by HPLC.

1.5 Determination of browning

For this experiment, 2 mL sample and 2 mL absolute ethanol were mixed in a 14 mL centrifuge tube, and centrifuged at 4000 r/min at 15 ℃ for 15 min. The absorbance at 420 nm was measured using a UV-160 ultraviolet-visible recording spectrophotometer (Shimadzu, Japan), diluted with 50% ethanol aqueous solution when required. Browning was expressed as the color value, which was calculated as the OD_{420} × dilution factor/reaction volume.

1.6 Determination of acrylamide and HMF

Acrylamide and HMF were determined by an Agilent 1200 Series HPLC System (Waldbronn, Germany) equipped with a diode array detector and Agilent Zorbax SB-Aq C_{18} column (4.6× 150 mm, 5 μm) according to our previously published method[12]. The injection volume was 5 μL. Elution was carried out at a flow rate of 0.5 mL/min under isocratic conditions at 30 ℃, using deionized water as the mobile phase. Acrylamide and HMF were detected at 205 and 284 nm respectively, and quantified using their calibration curves.

2 RESULTS AND DISCUSSION

2.1 Effect of whole-grain flour on the formation of acrylamide and HMF

Addition of 0.2 g whole-grain flour to the model reaction system influenced the content of acrylamide, and a great difference was found among the various varieties (Table 1). Black soybean, yellow soybean, yellow pea, mungbean, and lentil significantly decreased the formation of acrylamide (reduced by 10.3% (lentil) to 55% (black soybean). However, chickpea and green pea flour slightly increased the formation of acrylamide. Compared to their effect on the formation of acrylamide, the whole-grain flours from four of the seven tested legumes significantly increased the formation of HMF. The other three also slightly increased the formation of HMF (Table 1). Besides black soybean and green pea which contain pigments in the seed coats, addition of the whole-grain flour of all other legumes decreased browning after the Maillard reaction (Table 1).

2.2 Effect of defatted whole-grain flour on the formation of acrylamide and HMF

Table 1 Effects of whole-grain legume flours (0.2 g added before and after defatting) on browning and formation of acrylamide and HMF

Treatment	Acrylamide /(μg/tube)	HMF /(μg/tube)	Color value (OD_{420}/mL)
Control	270.1±19.3^{Fa}	52.2±2.0^{A}	6.26±1.00^{EF}
Black soybean	121.7±6.9^{B}	149.8±4.3^{F}	7.36±0.41^{FG}
Chickpea	288.5±13.1^{G}	114.2±0.4^{CD}	5.11±0.10^{CD}
Green pea	310.5±6.4^{G}	127.5±12.4^{E}	7.18±0.47^{FG}
Lentil	242.2±7.2^{F}	78.8±4.4^{B}	4.99±0.09^{CD}
Mung bean	225.5±4.6^{E}	54.8±2.8^{A}	5.72±0.51^{DE}
Yellow pea	213.5±3.8^{E}	54.0±1.8^{A}	6.22±0.62^{EF}
Yellow soybean	142.2±7.3^{C}	58.0±2.8^{A}	5.18±0.97^{CD}
Defatted black soybean	143.7±4.3^{C}	137.5±3.3^{E}	6.78±0.64^{FG}
Defatted chickpea	141.1±7.4^{C}	119.6±9.7^{C}	5.18±0.03^{CD}
Defatted green pea	97.4±14.9^{A}	103.0±6.7^{C}	3.48±0.19^{A}
Defatted lentil	174.0±6.7^{D}	87.0±3.6^{B}	3.58±0.25^{A}
Defatted mung bean	143.0±20.0^{C}	116.3±8.8^{D}	4.46±0.10^{BC}
Defatted yellow pea	117.9±19.1^{B}	87.3±1.4^{B}	4.03±0.02^{AB}
Defatted yellow soybean	141.4±5.2^{C}	102.3±2.6^{C}	5.15±0.30^{CD}

Note: ^{a}Means±SD (n=3) with different capital letters within a column are significantly different at the 5% level.

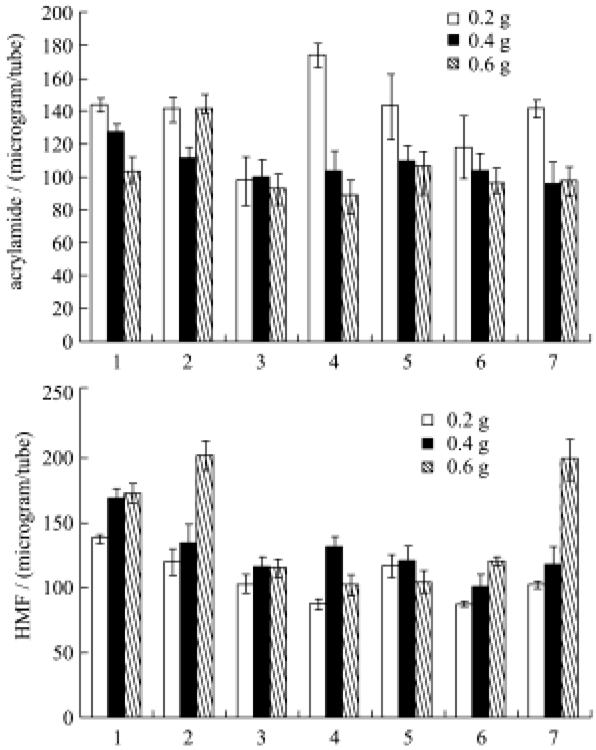

Fig.1 Effects of different amounts of defatted legume flours on formation of acrylamide (above) and HMF (below)

Note: 1 to 7 in the X axis refers as black soybean, chickpea, green pea, lentil, mung bean, yellow pea, and yellow soybean respectively.

When the whole-grain flours were defatted, all the samples showed an inhibitory effect on the formation of acrylamide (Table 1), and all of them-except black soybean flour-showed higher capacity than their undefatted ones for reducing the formation of acrylamide (Table 1), from 35.58% (defatted lentil) to 63.91% (defatted green pea). Oil oxidation may play a role in the formation of acrylamide. Under conditions of high moisture and high temperature, the oil in the undefatted flour is oxidized to form carbonyl compounds, which have been shown to have a higher activity than reducing sugars in promoting the formation of acrylamide[13]. Some phenolic compounds (especially chlorogenic acid) presented in black soybean are partially removed during the defatting process; the chemical substances may contribute to the inhibition of the formation of acrylamide, for some antioxidants at higher concentrations are thought to decrease acrylamide content [8]. As discussed later, anthocyanin-rich hulls from black soybean, shows high inhibition capacity toward formation of acrylamide compared to the other parts. The formation of HMF was significantly increased by addition of four varieties of legume flour (lentil, mungbean, yellow pea and yellow soybean), but browning appeared to be reduced by the addition of defatted flours compared with the results from the respective undefatted ones (Table 1). The formation of acrylamide significantly decreased with the addition amount of defatted flour in the model reaction system (Fig. 1). However, all of the deffated legume flours apart from black soybean further increased the HMF content (Table 1). Increased the addition of legume flours increased HMF formation by black soybean, chickpea, yellow soybean, but addition of 0.6 g/tube lentil flour decreased HMF production compared with the control (Fig.1).

2.3 Effect of defatted-dehulled legume seed flour on formation of acrylamide and HMF

Table 2 Effects of defatted- dehulled legume seed flours on formation of acrylamide and HMF

	Black soybean	Chickpea	Green pea	Mungbean	Yellow pea	Yellow soybean
Acrylamide/(μg/tube)	123.7±2.4^{Ca}	123.8±2.8^{C}	75.8±7.2^{A}	67.3±7.2^{A}	99.9±2.5^{B}	99.6±3.6^{B}
HMF/(μg/tube)	162.5±1.8^{D}	208.2±7.5^{B}	144.5±7.8^{C}	110.8±12.2^{B}	156.3±4. 6^{C}	83.4±2.8^{A}

Note: ^{a}Means±SD(n=3) with different capital letters within a line are significantly different at the 5% level.

To investigate whether the seed coats of the legumes influenced the formation of acrylamide and HMF, 0.2 g the defatted-dehulled seed flours (except for lentil, in which case the seed hull is difficult to remove by hand) were added to the model reaction system. The results showed that conten of acrylamide was further reduced, but HMF was increased in the treatments when using black soybean, green pea, chickpea, and yellow pea, by 13.1%, 48.4%, 47.6% and 32.6% respectively with the defatted ones (Table 2).

2.4 Effect of the seed hull on the formation of acrylamide and HMF

Both positive and negative effects of antioxidants on the formation of acrylamide have been reported[8]. It has been assumed that high levels of antioxidants might form quinones derivatives, which are able to directly destroy acrylamide and its precursor-asparagine, thus inhibiting the formation of acrylamide. But some antioxidants at low level were reported to increase the content of acrylamide[8]. The seed coat of some legume seeds contains much higher levels of phenolic antioxidants than the seed flour[10]; which may influence the formation of acrylamide. In this research, 0.2 g of the hull from four varieties (the amount of added hull here is more than 10 times equivalent of the whole-grain seed flour because the hull only makes up approximately 8% of the whole seed) showed inhibition capacity for formation of acrylamide but promoted the generation of HMF (Table 3). Moreover, much deeper browning occurred after addition of the hull compared with the results obtained using seed flour (Tables 1 and 4), indicating that phenolic compounds directly influenced browning, because the hull contained very little amounts of reducing sugars and free amino acids.

Table 3 Effects of legume seed hulls on the formation of acrylamide and HMF

	Black soybean	Chickpea	Green pea	Yellow soybean
Acrylamide/(μg/tube)	114.9±9.6^{Aa}	206.9±8.4^{D}	183.2±7.3^{C}	132.9±11.0^{B}
HMF/(μg/tube)	233.8±11.2^{B}	160.4±16.7^{A}	149.7±7.4^{A}	211.5±22.7^{B}
Color value (OD_{540}/mL)	17.69±0.49^{C}	15.18±0.98^{B}	10.94±1.12^{A}	14.68±1.95^{B}

Note: ^{a}Means±SD(n=3) with different capital letters within a row are significantly different at the 5% level.

Table 4 Effects of defatted legume seed flours on elimination of acrylamide

	Control	Black soybean	Chickpea	Green pea	Lentil	Mungbean	Yellow pea	Yellow soybean
Acrylamide/(μg/tube)	92.3±0.6^{Fa}	78.7±2.8^{CD}	76.8±2.9^{C}	84.4±1.1^{DE}	73. 8±3.0^{B}	86.9±0.7^{EF}	92.3±3.76^{F}	66.0±3.8^{A}

Note: ^{a}Means±SD (n=3) with different capital letters within a line are significantly different at the 5% level.

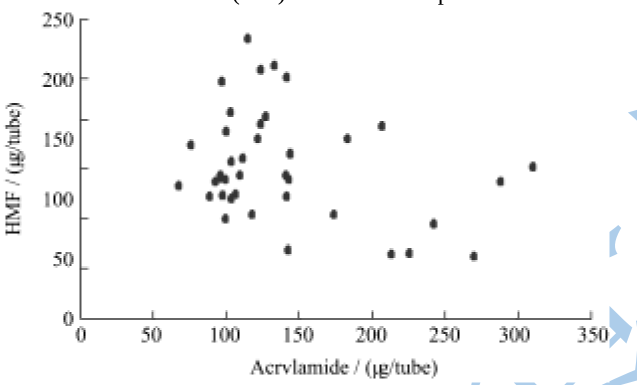

Fig.2 Contents of acrylamide versus HMF in the 39 treatments studied in this research

2.5 Effect of defatted legume seed flour on the elimination of acrylamide

Addition of 0.2 g the defatted legume flour to an acrylamide solution and subsequently heating at 160 ℃ for 15 min showed the capacity of all legumes, except for yellow pea, an elimination of acrylamide(Table 4). And the elimination of acrylamide was ranged from 5.9% (mungbean) to 28.5% (yellow soybean). The elimination of acrylamide may be due to the high lysine content in the legume protein. Lysine can eliminate acrylamide through the Michael reaction. However, this may not be the sole mechanism, since green pea contained much higher lysine than chickpea seed[14], but eliminated less acrylamide than chickpea. We attributed this effect partly to the existing antioxidants, the oxidized derivatives of which would destroy acrylamide[8], because the legume seeds with higher phenolic contents, namely, black soybean, lentil, and chickpea[10], showed significantly higher elimination capacity for acrylamide. However, the elimination percentages were below the reduction percentages obtained with defatted flour in the model reaction system, as shown in Table 1. The most effective elimination varieties (lentil and the two soybeans) were not the most inhibitory ones (which were defatted green pea and yellow pea in Table 1) with reference to the formation of acrylamide, indicating that the legume flours still have routes other than elimination to reduce acrylamide content in the model system.

3 CONCLUSION

Legume flours inhibited the formation of acrylamide but increased the formation of HMF, with significant differences existing among the different varieties. The inhibitory capacity of legume flours on the formation of acrylamide in the model reaction system used in this research shows their practical application in the reduction of acrylamide content in foodstuffs. Legumes flours have advantages over meat proteins in terms of providing valuable dietary proteins. Legumes are thermal stable, easy to prepare and incorporate in various food applications, and do not produce other toxic substances, such as heterocyclic amines[15], which are more active carcinogens than acrylamide, and may be produced during high-temperature processing of meat proteins because of the presence of creatine.

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