PRODUCTION OF VALUE ADDED PRODUCTS FROM SOME CEREAL MILLING BY-PRODUCTS

By

SAYED SAAD ABOZAIED SMUDA

B.Sc. Agric. Sci. (Food Science), Fac. Agric., Cairo Univ., 2004
M.Sc. Agric. Sci. (Food Science), Fac. Agric., Cairo Univ., 2013

THESIS

Submitted in Partial Fulfillment of the
Requirements for the Degree of

DOCTOR OF PHILOSOPHY

In

Agricultural Sciences
(Food Science)

Department of Food Science
Faculty of Agriculture
Cairo University
EGYPT
2018

SUPERVISION SHEET

PRODUCTION OF VALUE ADDED PRODUCTS FROM SOME CEREAL MILLING BY-PRODUCTS

Ph.D. Thesis

In

Agric. Sci. (Food Science)

By

SAYED SAAD ABOZAIED SMUDA

B.Sc. Agric. Sci. (Food Science), Fac. Agric., Cairo Univ., 2004
M.Sc. Agric. Sci. (Food Science), Fac. Agric., Cairo Univ., 2013

SUPERVISION COMMITTEE

Dr. SOBHY MOHAMED MOHSEN
Professor of Food Science, Fac. Agric., Cairo University

Dr. MOHAMED HASSAN ALY
Associate Professor of Food Science, Fac. Agric., Cairo University

Dr. KARSTEN OLSEN
Associate Professor of Food Science, Fac. Sci., Copenhagen University

Name of Candidate: Sayed Saad Abozaied Smuda **Degree:** Ph.D.
Title of Thesis: Production of value added products from some cereal milling by-products
Supervisors: Dr. Sobhy Mohamed Mohsen
Dr. Mohamed Hassan Aly
Dr. Karsten Olsen
Department: Food Science **Branch:** Food Industry **Approval: 28 / 3 / 2018**

ABSTRACT

The present study was performed to evaluate the phytochemicals profiles proximate composition, functional characteristics and certain anti-nutritional factors in some cereal milling by-products such as wheat (bran, germ and shorts), rice (bran, germ and husk) and corn (bran, germ and germ meal) to assess their potentiality as source of protein, fat and dietary fiber as well as bioactive compounds. In addition, distilled water, ethanol, methanol, and acetone separately were used as a various solvents in the extraction of phytochemicals compounds. The antioxidant activity (AOA), total phenolics content (TPC), and total flavonoids content (TFC) of the extracts were investigated using various in vitro assays. The major findings of this study are as follows: crude protein ranged from 10.45- 29.90%, fat 3.90 -47.53%, total dietary fiber 20.20-41.13%, bulk density 0.25-0.79 g/ml, water absorption 122.71-510.02 g/100g, fat absorption 89.51-235.12 g/100g and free fatty acids 6.75-19.94%. Significant variations were observed with regard to the color of different cereal milling by-products. The results showed that tannins content was ranged from 113.4-389.5 (mg /100g sample).The study revealed that the TPC and TFC of cereal by-products extracts were significantly different by using various solvents ($P<0.05$), which TPC content varied from 366.1 to 1924.9 mg /100gm and TFC content varied from 139.3 to 681.6 mg/100gm. High carotenoids content was observed in corn germ meal and minimum in wheat bran. Distilled water, ethanol and methanol extracts showed significantly different in antioxidant activity. Significant variations observed with regard to AOA of different cereal by-products by using various solvents. The results concluded that ethanol and methanol were the best solvents to extract phenolic compounds and antioxidant activity, while acetone extracts reported less efficiency. Results also revealed that vitamin E content was varied from 175.50 to 335.73 μg/g of different cereal milling by-products. Cereal milling by-products i.e. wheat, rice and corn brans were processed into Nano-bioactive freeze drying extracts. The results showed that the antioxidant activity (AOA) (the efficiency of AOA) of Nano-freeze drying ethanolic extracts of were highly significantly different compared to cereal brans extracts being 412.90, 318.84 and 205.34 % RSA compared to 70.00, 59.00 and 49.00 % RSA for normal sample extract for corn, rice and wheat brans, respectively. The results also concluded that the addition of Nano-bioactive of cereal bran improved the oxidative stability of the emulsions.

Keywords: Phytochemicals ; Total phenolics content; Antioxidant activity; Cereal milling by-products; Anti-nutritional factors

SUMMARY

The present work was conducted to study the different cereal milling by-products generated during milling process of wheat, rice and corn as well as their chemical compositions, nutritive values, phytochemical profiles and antioxidant activities. The cereal milling by-products were identified into i.e. (bran, shorts and germ of wheat milling, bran, germ and husk of rice milling, bran, germ and germ meal of corn milling). The determination of phytochemicals profiles and dietary fiber composition of cereal milling by-products was also studied as well as evaluation of their antioxidant activity. In addition, the utilization of cereal milling by-products as a value added products (as a source of dietary fiber, bioactive substances and natural antioxidant activities) were evaluated. Moreover, the cereal brans Nano-bioactive ethanolic extracts were prepared from cereal brans (i.e. wheat, rice and corn) and evaluated for their yield and antioxidant activity. In addition, the Nano-bioactive compound extracts were added to oil/water emulsion and kinetic stability were studied during the storage period. The results of the present study could be summarized in the main following points:

1. Nutritive value of cereal milling by-products

a. Chemical composition of cereal milling by-products

Results showed that the wheat germ was rich in protein (29.90%), fat (10.69%) and total sugars (5.50%).Wheat bran was rich in protein (16.67%), fiber (9.68%) and total soluble sugars (4.18%). The results also revealed that rice germ was rich in fat (25.43%), protein (17.34%) and total soluble sugars (5.09%). Rice bran was rich in fat (20.47%), protein (13.98%) and fiber (13.39%).

Concerning corn by-products, results showed that corn germ meal was rich in protein (27.78%) followed by germ (14.32%) then bran (12.86%). While corn germ was rich in fat (47.53%) followed by germ meal (23.60%) and bran (3.90%). Therefore, the results concluded that wheat germ (29.90%), corn germ meal (27.78%), wheat bran (16.67%), rice germ (16.37%) and corn germ (14.32%) could be utilized as a source of protein for different food products. In the same line, corn germ, rice germ and corn germ meal could be used as a source of fat.

b. Minerals content of cereal milling by-products.

The results revealed that the cereal that Potassium was found to be the most abundant mineral followed by magnesium. While, manganese was the lowest amount of minerals content.

Results also showed that wheat germ, rice husk and corn germ meal were the highest cereal milling by-products in minerals content.

This indicates that cereal milling by-products i.e. germ, bran and husk could be used as good source of minerals i.e. Fe, Zn and Mn that cover all the recommended dietary allowance (RDA) of Fe, Zn and Mn.

c. Dietary fiber composition and fiber fractions of cereal milling by-products.

The results indicated that rice husk significantly had the highest percentage of total dietary fiber (41.13 %) followed by corn bran (35.40%), wheat bran (33.90%), rice bran (33.12%), rice germ (29.74%), wheat shorts (29.48%), wheat germ (28.05%), corn germ meal (24.50%) and corn germ (20.20%). The results revealed that the rice husk had the highest value in dietary fiber yield, total dietary fiber, and insoluble dietary fiber. In addition, corn germ had the lowest percentage of total dietary fiber, insoluble dietary fiber.

The results indicated that rice husk significantly had the highest amount of cellulose (17.32%) followed by corn bran, rice bran, wheat bran, rice germ, corn germ meal, corn germ and wheat germ (14.25%, 12.97%, 12.40%, 10.18%, 10.12, 9.43 and 8.52%, respectively). The results also revealed that rice husk had the highest percent of lignin (6.03%) followed by corn bran (5.73%), wheat bran (5.16%), rice bran (4.55%), wheat shorts (3.94%), corn germ meal (3.23%), wheat germ (3.10%) and corn germ (2.23%).

2. Functional characteristics of cereal milling by-products

Bulk density of particular corn milling by-products ranged between 0.25g/ml (corn bran) to 0.88g/ml (corn germ meal). Wheat milling by-products were ranged from 0.37 to 0.46 g/ml and rice milling by-products were ranged from 0.36 to 0.79 g/ ml.

Significant differences was observed with respect to water absorption of cereal milling by-products. Corn germ meal had the highest water absorption (510.02%), while wheat germ had the lowest water absorption (122.71%). The results also revealed that wheat bran significantly had the highest water activity. On the other hand, corn germ meal showed the lowest value of water activity.Significantly, the lowest percentage of free fatty acids was found in wheat shorts. The rice germ exhibited significantly higher values of free fatty acids as compared to other cereal milling by-products.

Lightness value for wheat, rice and corn by-products was ranged from 44.64 to 73.19. The results also showed that wheat germ had the highest value of the lightness (73.19) followed by rice bran (72.01) and corn bran (71.59). Corn germ meal and corn germ had more redness (12.02 and 11.30) followed by wheat bran. b* values (yellowness) was highest in corn germ (32.21), while the lowest valued was observed in wheat shorts (16.25).

3. Anti-nutritional factors of cereal milling by-products

Results showed that the corn germ meal had significantly the highest amounts of phytic acid followed by wheat germ and rice germ (4005.05, 3907.17 and 3713.40 mg/100g, respectively). The results also found that rice bran; corn germ and wheat shorts had significantly the lowest amounts of phytic acid.

The results concluded that corn bran had the highest content of trypsin inhibitor that ranged from 30.37-174.37 TIU/g.

The results also revealed rice husk was the higher level of oxalates (0.478 %) followed by rice bran (0.453 %), corn bran (0.434 %), wheat bran (0.416 %), wheat shorts (0.414 %), corn germ meal (0.346 %), wheat germ (0.326 %), rice germ (0.315 %) and corn germ (0.313 %).

Corn bran had higher saponins content (4.53 mg/g) followed rice husk (4.10 mg/g), then rice bran (3.71 mg/g), wheat germ (3.08 mg/g), wheat bran (3.02 mg/g), rice germ (2.89 mg/g), wheat shorts (2.68 mg/g) and corn germ (2.36 mg/g).

This means that the utilization of cereal by-products for human nutrition may need some processing treatments such as cooking, drying, microwave heating or chemical treatment to remove or eliminate the undesirable components.

4. Phytochemicals compounds of cereal milling by-products

The cereal milling by-products were analyzed for valuable phytochemicals such as total phenolics, total flavonoids, total carotenoids and tannins contents.

a. Total phenolics content (TPC)

The total phenolics content (TPC) of cereal milling by-products extracted with various solvents i.e. distilled water, ethanol, methanol and acetone were evaluated and expressed as mg Gallic acid Equivalent (GAE)/100g sample.

Results indicated a wide variation in the TPC in the different extracts of cereal milling by-products. The highest amount of TPC was obtained by using distilled water for wheat milling by-products (germ, bran and shorts being 1861.9, 844.7 and 698.2 mg GAE/100g, respectively) and corn bran being (1925 mg GAE/100g) followed by methanol (1814.0 mg GAE/100g), ethanol (1779.5 mg GAE/100g) and acetone (1538.0 mg GAE/100g). On the other hand, the lowest amount of TPC was achieved by using acetone. The results revealed that TPC of rice milling by-products (husk, germ and bran) and corn milling by-products (germ and germ meal) which extracted by ethanol were significantly high compared to methanol, distilled water and acetone, respectively.

b. Total Flavonoids content (TFC)

The results showed that TFC of cereal milling by-products extracted by acetone had the highest values followed by ethanol and methanol, respectively. On the other hand, distilled water had less efficiency for TFC extraction.

c. Carotenoids

The total carotenoids of cereal milling by-products were extracted by n-butanol and expressed as β-carotene.

The present study shows a significant difference between cereal milling by-products in carotenoids. Corn germ meal showed the highest content of carotenoids (57.86 µg/g) followed by corn bran (31.94µg/g). On the other hand, wheat bran showed the lowest value of carotenoids (4.16 µg/g).

d. Tannins content

The results revealed that corn germ meal significantly contained the highest value being (389.47 mg CE/100g) followed by corn germ and wheat bran (315.32 and 257.19 mg CE/100g, respectively) when compared with those of the rest of the tested cereal by-products. On the other hand, rice bran contained the lowest values being (113.37 mg CE/100g).

e. Antioxidant Activity (AOA)

DPPH, $ABTS^{+}$ radical scavenging activity and ferric reducing power (FRAP) of the various cereal milling extracts were determined. The extracts of each cereal milling by-products were evaluated for their free radical scavenging properties.

The data showed that antioxidant activity of different cereal milling by-products which determined by different Antioxidant Activity (AOA) methods such as DPPH, $ABTS^+$ and FRAP were significantly different according to different solvent extracts ($p< 0.05$).

Concerning wheat by-products, results showed that germ had higher values of AOA compared to bran and shorts. The results also revealed that methanol was more efficient in the extraction of bioactive compounds of high antioxidant activity.

The results revealed that rice bran significantly had the highest values in TPC and TFC and had antioxidant activity ranged from 47.3 to 65.1 % RSA as DPPH assay, 35.4 to 74.3 % RSA as $ABTS^+$ assay and 20.6 to 40.3 mmol L^{-1} ferrous sulfate/g as FRAP assay. Ethanol and methanol were more efficient in the extraction of antioxidant compounds from rice milling by-products.

Concerning corn milling by-products; i.e. bran, germ and germ meal, the results revealed that AOA extracts by various solvents were ranged from 37.4 to 60.5, 49.8 to 70.0, 52.1 to 71.3 and 29.8 to 40.3 % RSA as DPPH assay for distilled water, ethanol, methanol and acetone, respectively. This means that ethanol and methanol were more efficient in the extraction of bioactive compounds of high antioxidant activity.

These data suggested that ethanol and methanol were the best solvents to extract phenolic compounds and antioxidant activity. Therefore, ethanol and methanol might be a better choice for extracting antioxidants compounds from cereal milling by-products samples.

f. Vitamin E (Tocopherols and Tocotrienols profiles of cereal milling by-products)

Results showed that wheat germ and rice husk had the highest alpha-tocopherol value (54.98 µg/g) than other cereal milling by-products. Moreover, wheat shorts contained the highest contents of beta-tocopherol (58.28 µg/g) and alpha- tocotrienol (25.68 µg/g).

Results also showed that the highest content of gamma-tocopherol was observed in corn germ meal (99.90 µg/g). While, rice husk was the highest cereal milling by-products in gamma- tocotrienol (112.34 µg/g). Among cereal milling by-products, wheat bran had the highest values of delta- tocotrienol (99.99 µg/g).

Results also indicated that vitamin E content was varied from 175.50 to 335.73 µg/g of different cereal milling by-products.

It could be noticed that wheat bran (335.73 µg/g) and wheat germ (325.90 µg/g) followed by rice husk (299.90 µg/g), corn germ meal (288.90 µg/g) and corn bran (276.17 µg/g) were founded to be a rich sources of vitamin E (tocopherols and tocotrienols).

g. Identification of phenolic compounds

Phenolic compounds of cereal milling by-products were extracted and fractionated by HPLC. Ferulic acid was the predominant phenolic acid in all cereal milling by-products samples.

Concerning rice milling by-products, rice germ and husk contained the highest values of most fractionated phenolic compounds then followed by rice bran. In addition corn milling by-products contained the highest level of *p*-hdroxybenzoic acid, vanillic acid, cinnamic acid, salicylic acid, ellagic acid and benzoic acid than those of wheat and rice milling by-products. This means that these cereal milling by-products are rich sources of phenolic compounds.

5. Utilization of cereal milling by-products as a value added products

As mentioned before all cereals milling by-products were determined and identified in different produced by-products. The identified materials showed the availability of their use as a source of dietary fiber, bioactive compounds as well as antioxidant activities. To improve their efficiency, characteristics and values an essential nutrients for the production of consumer health, the Nano-bioactive products were carried out and evaluated.

a. Production and characterization of Nano-bioactive freeze drying extracts

Cereal milling by-products i.e. wheat, rice and corn brans were processed into Nano-bioactive freeze drying extracts. The results showed that the optimum parameters to produce Nano-bioactive compounds (112 – 214 nm) from cereal brans were 400 watt for 20 min at 50 ^{0}C.

The results showed that grinding of bran followed by ultrasonication treatment (400 watt for 20 min at 50 ^{0}C) increased the extraction yield of bioactive compounds by 43.9, 47.2 and 31.40 % of wheat bran, rice bran and corn bran Nano-bioactive freeze dried extracts, respectively. The results also revealed that TPC and TFC were increased by 47.7 and 40.8, 39.8 and 48.9, 32.4 and 66.1 % for wheat bran, rice bran and corn bran Nano-bioactive freeze drying extracts, respectively. This increase could be attributed to grinding and sonication treatment that caused more break down to the cell walls.

The results showed that the antioxidant activity (AOA) (the efficiency of AOA) of Nano-freeze drying ethanolic extracts of phytochemicals were highly significantly different compared to cereal brans extracts. The phyto-nanoparticles exhibited higher antioxidant activity compared to normal particles of the plant extract. In addition, the antioxidant activity in Nano-bioactive freeze drying extracts of cereal brans were carried out using free radical DPPH method and expressed as IC_{50} values. The inhibitory concentration at 50% (IC_{50}) values (extract concentration that cause 50% scavenging of DPPH radical) were evaluated and compared with natural and artificial controls ascorbic acid (AA) and butylated hydroxyanisole (BHA).

The results showed that ascorbic acid had the least value IC_{50} (8.23 µg ml^{-1}) followed by Nano-freeze drying extract of corn bran (10.34 µg ml^{-1}) indicating higher bioactive compounds and antioxidant activities compared to the Nano-freeze drying extracts of rice and wheat brans (15.84 and 21.90 µg ml^{-1}), respectively. On the other hand, the results showed that wheat bran Nano-bioactive freeze drying extract had the highest value of IC_{50} followed by BHA as artificial positive control. This means that cereal brans extracts could be used as a potential source of antioxidants activities.

b. Production and characterization of Nano-Emulsion

Nano-emulsions were prepared containing 2% sunflower oil and tween 80 as surfactant incorporated in Nano-freeze drying ethanolic extracts (0.2%) of different cereal brans as natural antioxidants. The physicochemical properties of each Nano-emulsion was characterized and compared with positive and negative control (ascorbic acid Nano-emulsion and free bioactive compounds Nano-emulsion), respectively. In addition, the Nano-emulsions were evaluated for its physical and chemical stability during storage at 50 ^{0}C for 25 days.

1. **Oxidative stability**
   a. **Lipid Hydroperoxides**

The results showed an increase in lipid hydroperoxides concentration was observed up to 15 days upon storage in oven at 50 ^{0}C, then decreased until 25 days for all prepared emulsions.

The results showed that at the end of storage period oxidation products of ascorbic acid was the lowest value emulsion followed by rice bran emulsion and corn bran emulsion then wheat bran emulsion were lower than the control (free bioactive compounds). These results suggest a possible antioxidant effect of Nano-emulsion supported by Nano-bioactive freeze drying extracts of corn and rice as a natural antioxidant compounds.

b. **Inhibition of lipid oxidation in emulsions**

All the ethanolic Nano-freeze drying extracts of different cereal brans showed inhibitory effects towards oxidation of the emulsified corn oil. Under the conditions employed in this study, the presence of wheat bran extract led to a decrease in oxidation to a final 50.3 % with respect to the degree of oxidation observed in emulsions with negative control (Emulsion free bioactive extract).

The ascorbic acid showed additional decreases in degree of oxidation (33.8%); and when using Nano-freeze drying extracts of RB and CB, the oxidation were only 32.9 and 30.0% relative to the degree of oxidation with negative control (Emulsion free bioactive extract). This means that Nano-bioactive freeze drying extracts of rice and corn brans had a high antioxidant activity similar to the results obtained by using ascorbic acid.

2. Cloudiness or Turbidity

The results showed that free bioactive compound control (negative control) was more stable than all tested emulsions. On the other hand, wheat bran emulsion showed less stable during the storage period than other emulsions.

3. Mean droplet size (Z-Average) measurements

The size distribution of droplets in the oil/ water emulsions containing Nano-freeze drying extracts of cereal brans were measured based on laser light scattering. Average droplet sizes of dispersed oil droplets one day after Nano-emulsion production were 275.6, 301.6, 318.0, 279.1 and 338.0 nm for corn bran, ascorbic acid, wheat bran, rice bran and negative control emulsions, respectively. The results also observed an increase in droplet size to 468.6, 353.4, 455.8, 375.0 and 359.0 nm at 25 days after storage of negative control, ascorbic acid as positive control, wheat bran, rice bran and corn bran emulsions, respectively. The results also showed that the emulsions maintained its stability during storage and no sedimentation was observed.

4. Emulsion stability Index (ESI)

The creaming stability of the emulsions was evaluated by determining the phase separation over time after storage time for 25 days at 50 ^{0}C.

The results showed that emulsion stability index (ESI) of the wheat bran (WBE) 96.34 % and control (CE) 94.50% emulsions had the highest values followed by corn bran emulsion (CBE) 90.50 %, ascorbic acid emulsion (AAE) 85.00 % then rice bran emulsion (RBE) 76.70%.

The emulsions without Nano-bioactive freeze drying extracts as a negative control and with Nano-bioactive wheat bran extract were more stable (25 days of storage), moreover the oil layer separated on top was found to be 5.5 and 3.7 % for wheat bran emulsion and the negative control, respectively. Also the emulsions showed that no oil layer on top was observed. This indicated that, the oil droplet coalescence in addition to creaming.

5. Size distribution measurements

The polydispersity index (PDI) of different Nano-emulsions measured by Malvern Zetasizer N90. The results showed that very little increase in polydispersity during the storage.

The results showed that polydispersity of the Nano-emulsions were increased during the storage from 0.318 to 0.499, 0.402 to 0.533, 0.331 to 0.601, 0.310 to 0.545 and 0.378 to 0.504 of control, ascorbic acid, wheat bran, rice bran and corn bran emulsions, respectively.

The results concluded that slight physically changes (i.e. size, turbidity creaming value) among Nano-emulsions with different Nano-bioactive freeze drying extracts. Moreover, the addition of Nano-bioactive freeze drying extracts from different cereal bran improved the oxidative stability of Nano-emulsions.