JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Micronutrient and Protein-Fortified Whole Grain Puffed Rice Made by Supercritical Fluid Extrusion

Ilankovan Paraman, Michael E. Wagner, and Syed S. H. Rizvi*

Institute of Food Science, Cornell University, 151 Stocking Hall, Ithaca, New York 14853-7201, United States

ABSTRACT: Supercritical fluid extrusion (SCFX) was used to produce shelf-stable puffed rice fortified with protein, dietary fiber, and micronutrients. Product ingredients and process parameters were evaluated for end-product nutritional and textural qualities. Supercritical carbon dioxide ($SC{-}CO_2$) served as a viscosity-lowering plasticizer and blowing agent during the process, which has been shown to produce expanded products with good textural qualities at lower temperatures (~100 °C) than conventional steam-based extrusion (130−180 °C). The fortified puffed rice contained 8% dietary fiber, 21.5% protein, and iron, zinc, and vitamins A and C at their recommended daily values in 100 g of product. The SCFX process allowed for the complete retention of all added minerals, 55−58% retention of vitamin A, and 64−76% retention of vitamin C. All essential amino acids including lysine were retained at exceptionally high levels (98.6%), and no losses were observed due to Maillard reaction or oxidation. All of the essential amino acid contents were equal to the reference protein recommended by FAO/WHO. Soy protein fortification improved the total amount of protein in the final rice products and provided a complementary amino acid profile to that of rice; the lysine content improved from 35 to 60 mg/protein, making the end product an excellent source of complete protein. Thus, $SC{-}CO_2$-assisted extrusion is an effective process-based approach to produce cereal grain-based, low-moisture (5−8%) expanded products fortified with protein and any cocktail of micronutrients, without compromising the end-product sensory or nutritional qualities. These products are ideally suited for consumption as breakfast cereals, snack foods, and as part of nutrition bars for school lunch programs. The balanced nutritional profile and use of staple crop byproducts such as broken rice makes these expanded crisps unique to the marketplace.

KEYWORDS: *supercritical fluid extrusion, nutrient fortification, nutrient enrichment, cereal grain products, puffed products, micronutrients*

INTRODUCTION

Micronutrient deficiencies and malnutrition pose serious health concerns in many parts of the world. Developing countries often have the highest prevalence, but lack of access to nutritious foods, physical conditions, and even environmental issues can contribute to nutritional deficiencies anywhere in the world, regardless of the economic conditions. Women, infants, and school age children are the most likely groups to suffer from micronutrient and vitamin deficiencies.[1,2] For instance, iron deficiency affects 1.62 billion people globally and ranks as the ninth greatest risk factor for global disease burden; vitamin A deficiency affects 127 million preschoolers despite several recent efforts to alleviate malnutrition.[1,2] Several process-based fortification strategies are already being utilized to combat these global concerns, such as surface coating, dusting, and extrusion. Surface coating and dusting are used to fortify micronutrients in whole rice kernels[3] or synthetic kernels.[4] Extrusion-based strategies can be used to produce novel fortified products from grain flours and other grain materials.[5−7]

Extruded products are an attractive delivery system for addressing nutritional deficiencies as they can be consumed as such (breakfast cereals, snacks) or incorporated into nutrient bars and consumed by targeted groups like preschoolers with a high degree of acceptance. Low-moisture extruded products are shelf-stable and are amenable to easy handling and extended storage at room temperatures, which is very advantageous, particularly in low-income countries. Additionally, extrusion can utilize cereal grain coproducts such as broken rice, flour, or bran to produce products enriched with required nutrients.[6,8] Extrusion can also increase the bioavailability of some nutrients, such as iron and protein, through the inactivation of antinutritional factors. However, the high-temperature, low-moisture, and high-shear conditions of conventional extrusion can destroy substantial amounts of heat-labile nutrients.[9]

In conventional steam-based extrusion, water serves a dual role. It acts like a plasticizer during melt formation in the extruder barrel and then as a blowing agent when it flashes into steam at the die exit.[10,11] Although steam-based extrusion is widely used in cereal processing, it is usually carried out under relatively intense processing conditions such as high temperature (>130 °C), high shear (>150 rpm screw speed), and low moisture (~10%), particularly when expansion is required.[12] Such extreme operating requirements are often very destructive to heat-sensitive ingredients, like certain proteins, vitamins, flavors, and bioactives.

Supercritical fluid extrusion (SCFX), a hybrid process developed by combining extrusion and supercritical fluid technologies, utilizes supercritical carbon dioxide ($SC{-}CO_2$) as a viscosity-lowering plasticizer and expansion/foaming agent.[10,11,13] $SC{-}CO_2$ is widely used in food and nonfood applications because of its relatively benign properties and mild

Received: August 8, 2012
Revised: October 9, 2012
Accepted: October 15, 2012
Published: October 15, 2012

dx.doi.org/10.1021/jf3034804 | *J. Agric. Food Chem.* 2012, 60, 11188−11194

supercritical conditions, $T_c = 31$ °C and $P_c = 7.38$ MPa.[14,15] In the SCFX process, SC−CO_2 quickly penetrates the dough/melt and dissolves into the aqueous phase due to its gaslike diffusivity. Upon depressurization through the die, it nucleates in the extrudate and produces highly expanded products of defined morphologies at temperatures below 100 °C.[13,14]

With the low shear and low temperatures used in SCFX, heat-sensitive materials experience minimal processing losses.[10,11] Furthermore, as compared to steam-based extrusion, SC−CO_2-assisted extrusion produces products with less explosive puffing, resulting in a smoother surface and more uniform internal cell structure.[16] The improved internal and exterior structures enhance not only the product sensory attributes but also the end-product chemical stability such as reduced moisture absorption, reduced oxidative loss, improved flavor, and nutrient retention.[17]

In the present study, the SCFX process was evaluated in the production of rice-based expanded products fortified with heat-labile micronutrients and proteins. The effects of the process on the retention of micro- and macronutrients and on product quality were evaluated and compared with commercial samples. Nutritional implications of the process-induced changes on product characteristics have also been investigated.

■ MATERIALS AND METHODS

Ingredients. Waxy rice flour (RF-WO01120) and rice bran were obtained from Sage V Foods (Freeport, TX). Soy protein concentrate (SPC) (Arcon F) and powdered lecithin were provided by ADM specialty products (Decatur, IL). Distilled monoglyceride was provided by Danisco ingredients (Kansas, MO). Vitamin/mineral premix for rice flour (FT121132) was provided by Fortitech (Schenectady, NY).

Extrusion Formulations. Three batches of 30 kg of dry ingredient formulations were used to produce the following products: puffed rice made from rice flour as the main ingredient (formulation #1); fortified puffed rice made with rice flour, soy protein, and rice bran as the main ingredients (formulation #2); and micronutrient and protein-fiber fortified puffed rice made from formulation #2 with added micronutrients (formulation #3). The ingredient compositions of the three formulations are given in Table 1. All of the ingredients were mixed for 9 min in a 0.14 m^3 ribbon blender (Littleford Day Inc., Florence, KY).

Supercritical CO_2 Extrusion. A pilot-scale Wenger TX-57 Magnum corotating, self-wiping, twin-screw extruder with a barrel diameter of 52 mm and length to diameter ratio (L/D) of 28.5:1 (Wenger Manufacturing, Sabetha, KS) was operated at a feed rate of 35 kg/h and screw speed of 120 rpm.[10] The extruder parameters and operating conditions are summarized in Table 2. The conditions were selected from preliminary studies conducted to achieve good end-product textural qualities (expansion, crispiness, and hardness) while preserving nutritional retention. Because native rice flour was used as a main ingredient, ~80 °C barrel temperature was required to gelatinize the starch to puff the rice with maximum expansion and crispiness. The barrel temperature in the first three barrel zones was maintained at ~80 °C by circulating steam through barrel jackets to gelatinize starch. The fourth and fifth barrel zones were cooled rapidly by circulating chilled brine (−10 °C) as indicated in Figure 1. A flow restrictor was used to maintain the die pressure at ~10 MPa (1500 psi).

Table 1. Feed Formulations of Control and Fortified Puffed Rice

	puffed rice formulation (% wet basis)		
ingredients	control	protein and fiber fortified	micronutrient and protein-fiber fortified
rice flour (waxy)	97.0	66.5	66.5
rice bran		8.0	8.0
soy protein conc.		22.5	22.5
distilled monoglycerides	1.0	1.0	1.0
lecithin	1.5	1.5	1.5
salt	0.5	0.5	0.5
micronutrient premix[a]			<0.1

[a]Micronutrient premix (FT121132, Fortitech, Schenectady, NY) was added in a ratio of 325 mg premix/100 g formulation as recommended by the supplier.

Table 2. SCFX Process Parameters for Puffed Rice Production

process parameters	process conditions
feed rate (kg/h)	35
water flow (% feed)	14
SC−CO_2 injection rate (kg/s)	7.6×10^{-5}
SC−CO_2 injection pressure (MPa)	8.27
die pressure (MPa)	10.34
screw speed (rpm)	120
average SME (kJ/kg)	165.5

A pilot-scale supercritical fluid system was used to generate and inject SC−CO_2 at a constant flow rate (7.6×10^{-5} kg/s) into the barrel through four valves located at L/D of 24. The SC−CO_2 was injected at a pressure of 1200 psi (8.3 MPa) to maintain a continuous flow of SC−CO_2 into the product melt.[10] The water was injected at a flow rate of 14% of the feed flow rate. The extrudate was forced through rice kernel-shaped openings, as shown in Figure 2, and cut by a knife rotating at 1020 rpm to form the final product. The cut product was collected on perforated trays and dried to ~5−8% moisture content in a forced air oven at 90 °C for 40 min. A small amount of sample was frozen immediately at −40 °C and subsequently freeze-dried for vitamin analysis.

The specific mechanical energy (SME) input into the dough was calculated from the following equation:[18]

$$\mathrm{SME} = 37.3\left(\frac{\%\ \text{extruder load}}{100}\right)\left(\frac{\text{extruder screw speed}}{306}\right)\left(\frac{3600}{\text{extruder feed rate}}\right)$$

where the extruder screw speed is in rpm (120 rpm), 306 rpm is the maximum extruder screw speed, 37.3 kW is the power input, and the extruder feed rate is in kg/h (35 kg/h). The average SME input was 165.5 kJ/kg. The literature values for various extruded products have been reported to range from 100 to 800 kJ/kg.[7,8,18,19]

Physical Characterization. The mechanical properties of the extruded products were measured by using a TA-XT2 texture analyzer operating with Texture Exponent 32 software (Micro Systems, Godalming, United Kingdom). The puffed rice was compressed to 80% of their average original diameter using a 35 mm compression plate at a test speed of 2 mm/s. The peak force (g) and initial gradient (N/s) of the force−time curve were recorded and analyzed to calculate the hardness and compressive modulus of the products, respectively.[20] An average of 15 samples was used to determine the hardness and compressive modulus.

Piece density was measured using the sand displacement method. The procedure was repeated five times for each set of samples.[21] The bulk density of the extruded puffed rice was measured by filling a container of known volume with the product.[16] Five replicates were done for each set of samples. The expansion ratio was calculated by dividing the cross-sectional thickness of the extrudate by the cross-sectional thickness of the die opening. An average of 12 samples was used to determine the expansion ratio of each set of samples. The color of the dry-blend and ground extrudate was measured with a

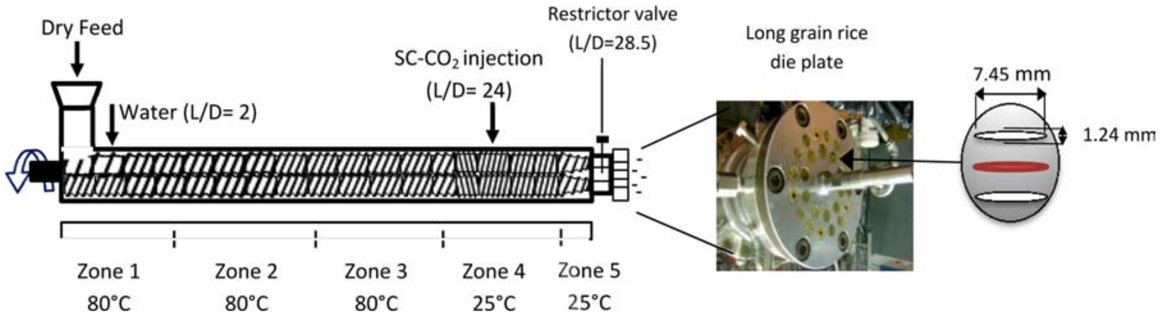

Figure 1. SCFX barrel and rice die configuration used in processing puffed rice.

Figure 2. Appearance of puffed rice products (A−C) made by SCFX and typical commercial puffed rice products (D−F). (A) SCFX made puffed rice, (B) protein-fiber fortified puffed rice, (C) micronutrient and protein-fiber fortified puffed rice, and (D−F) typical commercial puffed rice products made by conventional extrusion.

Table 3. Nutrient Composition of Ingredients and Puffed Rice Products

	ingredient			extruded rice		
nutrients	rice flour[a]	rice bran[b]	soy protein concn[c]	control SCFX puffed rice[d]	protein and fiber fortified SCFX puffed rice[e]	commercial puffed rice[f]
protein (%)	5.8	19.6	69	5.8	21.0	6.1
lipid (%)	0.8	4.8	2	0.8	1.4	0.6
dietary fiber (%)	0.9	39.3	19	0.9	8.0	0.9
carbohydrates (%)	82.9	51.5	20	82.9	63.7	87.9
ash (%)	0.3	8.5	7	0.3	2.5	0.3
energy (calories/100 g)	360	225	290	360	322.7	393.9
calcium (mg/100 g)	0	0.1	300	0	67.5	
phosphorus (mg/100 g)	0	1.7	800	0	180.1	
magnesium (mg/100 g)	0	0.7	200	0	45.1	
iron (mg/100 g)	1	2.3	8	1	2.6	
zinc (mg/100 g)	0	2.5	2	0	0.7	

[a] Supplier information. [b] Juliano.[24] [c] Supplier information. [d] Calculated based on ingredient composition. [e] Values based on formula #2 (no added micronutrients). [f] Quaker puffed rice.

HunterLab CIE $L^*a^*b^*$ system. Luminosity (L^*), red-green color opposition (a^*), and yellow-blue opposition (b^*) were determined.

Amino Acid Analysis. Amino acid profiles of the dry-blend and an extruded sample were determined at the Agricultural Experiment Station Chemical Laboratories, University of Missouri (Columbia, MO), by using AOAC Official Method 982.30 E (a, b, c), chapter 45.3.05.[22] Crude protein contents were determined by using the Dumas combustion method by AOAC Official Method 990.03.[22] The moisture content of the dry-blend and dried extrudates was measured by using oven drying method at 130 °C for 2 h (AACC 44-31).[23] The amino acid score was calculated using the FAO/WHO/UNU (1985) suggested pattern of amino acid requirement for preschool children (2−5 years).

Micronutrient Analysis. Iron and zinc concentrations of the dry-blend and ground extruded puffed rice were determined by using an inductively coupled argon-plasma/atomic emission spectrophotometer (ICAP 61 E Thermal Jarrell Ash Trace Analyzer, Jarrell Ash Co., Franklin, MA) after wet-ashing at the USDA plant soil and nutrition laboratory at Cornell University. Vitamins A and C in the dry-blends and extruded puffed rice were measured by Medallion Laboratories (Minneapolis, MN) by using AOAC 2001.13 method for Vitamin A and AOAC 967.22 method for vitamin C.[22]

Statistical Analysis. Extrusion trials were conducted in duplicate. Data were analyzed by analysis of variance using JMP 9.0.1 statistical software (SAS Institute Inc. 2010). The Tukey−Kramer HSD test was used to determine the least significant differences (LSD) at 5% significance level.

■ RESULTS AND DISCUSSION

Product Characteristics. The nutrient content of the feed stream and the resulting SCFX-generated puffed rice are given in Table 3. Also shown for comparison purposes in Table 3 is a typical composition of commercial puffed rice. The appearance of the puffed rice, protein, and fiber-fortified puffed rice, micronutrient-fortified puffed rice, and commercial puffed rice products are shown in Figure 2. As may be noted, by incorporating SPC and bran to produce the protein/fiber-fortified puffed rice, the protein content increased from 6.1 to 21.5% as compared to the control product (rice flour alone). Similarly, dietary fiber content also improved from 0.9 to 8%. The total calories of the fortified products decreased to 322 calories/100 g as compared to rice flour-alone products (360 calories/100 g) and commercial puffed rice products (393 calories/100 g). The protein and fiber fortification significantly enhanced the nutritive value of the final products. Whole grain brown rice (dehulled rice) is comprised of 8−10% bran.[23] Because 8% bran was incorporated in the extruded puffed rice in this study, the fortified puffed rice would satisfy the "whole grain" labeling requirements.

The physical characteristics of the SCFX processed puffed rice are given in Table 4. The puffed rice produced by SCFX using rice flour alone gave the best textural qualities. The products were very light in weight with 0.16 g/cm^3 piece density and expanded well with an expansion ratio of 5.0. When incorporating 8% rice bran and 22.5% SPC in the fortified puffed rice, certain textural attributes decreased as compared to the control puffed rice; expansion slightly decreased from 5.0 to 3.4, piece density increased from 0.16 to 0.31 g/cm^3, and hardness increased from 1403 to 2500 g force (Table 4). The limited expansion of the fortified puffed rice was mostly due to reduced gas-holding capacity of the dough caused by the presence of a significant amount of fiber and other insolubles in the added rice bran and SPC. Although proteins stabilize the air cells formed at the die exit,[16] the added rice bran could have reduced elasticity and CO$_2$-holding capacity of the dough. As a consequence, the final product expansion decreased, and the piece density and hardness increased.

Table 4. Physical Characteristics of SCFX Puffed Rice and Commercial Sample[a]

physical characteristics	control SCFX puffed rice[b]	fortified SCFX puffed rice[c]	commercial puffed rice[d]
piece density (g/cm^3)	0.16 b	0.31 a	0.12 c
bulk density (g/cm^3)	0.10 b	0.25 a	0.11 b
expansion ratio	5.0 a	3.4 b	
hardness (g)	1403.1 b	2500.5 a	1481.6 b
crispiness (number of peaks)	21.5 a	17.0 b	11.7 c
compression modulus (N/mm)	23.7 b	34.8 a	31.6 a

[a] Means in the same row followed by the same letter are not significantly different ($p < 0.05$). [b] Puffed rice made from rice flour as the main ingredient. [c] Protein-fiber fortified puffed rice made with rice flour, soy protein and rice bran as the main ingredients. [d] Quaker puffed rice.

However, the fortified puffed rice was very crispy and still showed considerable expansion and good overall textural characteristics. The crispiness of the products measured by both compressive modulus and number of peaks of the force−time deformation curve indicated that crispiness of the SCFX-processed fortified puffed rice were comparable to the control SCFX puffed rice. Both of the SCFX-processed products (control and fortified puffed rice) showed higher crispiness as compared to the commercial rice products (Table 4).

Vitamin Retention. Vitamin retention during SCFX process is shown in Figure 3. Fifty-five percent of the total vitamin A and 64% of vitamin C were retained after the SCFX process and postextrusion oven drying. Products that were freeze-dried immediately after extrusion rather than oven-dried retained 58% vitamin A and 76.7% vitamin C. Although vitamins A and C are sensitive to heat and oxidation, the low-temperature and low-shear conditions used in the SCFX process protected a significant portion (58−76%) of the vitamins. The losses were significantly lower than those reported in high-temperature steam-based extrusion. For instance, high barrel temperature (200 °C) with an 18.5% moisture content and 150 rpm screw speed reduced vitamin A in fortified wheat flour by 77%; however, on reducing the barrel temperature to 125 °C, the loss decreased to 48% under same processing conditions.[25] Depending on the severity of the process, a wide range of vitamin A loss 20−50% has been reported.[26,27]

Yoo et al.[7] reported 55% vitamin A retention in extruded rice kernels when using a traditional twin-screw extrusion process operating under high-moisture (30%) and moderate barrel-temperature (90 °C) conditions. The final temperature of the products at the die exit was not reported in this study. The process of Yoo et al.[7] retained only 28% vitamin C, which is much lower than that found in the present study. The SCFX-processed puffed rice retained 64% of the vitamin C, although vitamin C is the most sensitive of all vitamins to heat and oxidation.[9] Furthermore, the vitamin C retention was substantially higher (64−76%) than vitamin A (55−58%) retention; the reason for this may be loss to light-induced oxidation during the drying.

Mineral Retention. As minerals are heat stable, extrusion did not affect the mineral content of the fortified final products (Figure 4). However, extrusion processing can have an impact on mineral bioavailability. Although the present study did not measure the mineral bioavailability, it has been well documented that mineral bioavailability increases during extrusion due to inactivation of phytates and other antinutritional compounds.[28] However, the bioavailability of iron decreased when using high-temperature (180 °C) and -shear (200 rpm) conditions as compared to low-temperature (140 °C) and -shear (150 rpm) conditions used in lentil, chick pea, and cowpeas extrusion.[29] The extreme conditions oxidized the iron and reduced its ability to dialyze across the cell membrane. Therefore, the SCFX process using mild-temperature, shear

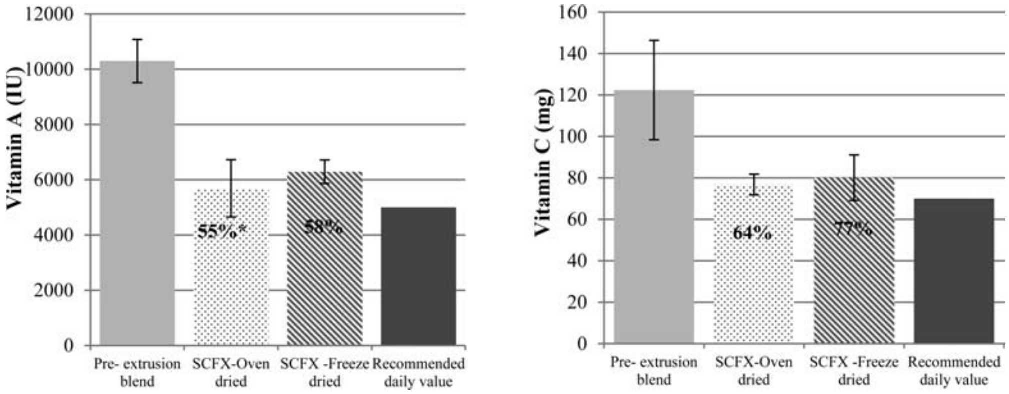

Figure 3. Vitamins A and C retention in fortified 100 g of puffed rice and their recommended daily values. *The numbers on the bar are the percent of vitamins retained after the SCFX process and postextrusion drying.

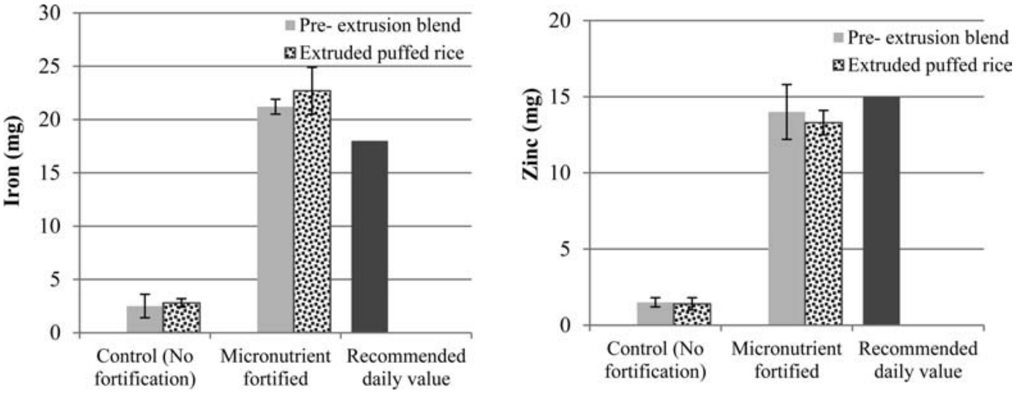

Figure 4. Iron and zinc retention in control and fortified puffed rice of 100 g and their recommended daily values.

conditions may improve the bioavailability of iron and other minerals without oxidizing them extensively. Further studies on mineral bioavailability of SCFX-processed products need to be conducted to confirm this concept.

Oxidation of iron or the interaction of minerals with other compounds during high thermal processing can darken the product color. However, color analysis of the SCFX-processed fortified puffed rice indicated that added micronutrients did not affect the color of the final products (data not shown), which again supported the idea that no undesirable oxidation reactions occurred during the SCFX process.

The dietary reference intake of micronutrients for different age groups and the amount of the micronutrient available per serving of the fortified rice product (100 g) are also shown in Figures 3 and 4 and Table 5. Higher levels of vitamins can be added to compensate the losses during the extrusion process, resulting in a product that satisfies the recommended daily values of 5000 IU vitamin A, 70 mg of vitamin C, 18 mg of iron, and 10 mg of zinc in a single 100 g serving.

Table 5. Recommended Dietary Reference Intakes for Iron, Zinc, and Vitamins A and C[a]

	recommended dietary reference intakes			
age, gender, and life stage group	vitamin A (μg/day)	vitamin C (mg/day)	iron (mg/day)	zinc (mg/day)
infants	500	50	11	3
children	400	25	7	5
adult men	900	90	8	11
adult women	700	75	18	8
pregnant	750	85	27	11
lactating	1300	120	10	12

[a] Adapted from NIH/Office of dietary supplements guidelines.

Lysine Retention. Table 6 shows the amino acid composition of fortified puffed rice made by SCFX. All of the amino acids were retained at very high level (96−100%) in the extruded products. Typically, high-temperature and low-moisture conditions promote Maillard reaction and oxidation of amino acids. Previous studies showed that significant amounts of lysine were lost due to Maillard reaction, and the loss ranged from 5 to 40% depending on processing conditions and ingredient composition used in conventional steam-based extrusion.[30] Lysine loss in extrusion is largely influenced by process temperature, moisture content, and the presence of other sugars. Because lysine is the first limiting essential amino acid in cereal grains, additional losses can further reduce the protein quality in final products. In the SCFX process, 98.6% of the total lysine was retained. SCFX processing combined with postextrusion oven drying and did not alter the amino acid profile of the proteins in the final products.

In addition to lysine, sulfur-containing essential amino acids are also lost in conventional high-temperature extrusion processing.[25] Kohler, 1981, as cited in Bjorck and Asp,[25] reported that, in addition to 30% lysine loss, significant amounts of other amino acids were lost, including 21% of arginine, 15% of histidine, 14% of aspartic acid, and 13% of serine during extrusion cooking at 160 °C temperature, 200 rpm screw speed, and 12% feed moisture. The amino acid losses not only reduced the amino acid contents but also

Table 6. Amino Acid Retention in Fortified Puffed Rice[a]

	AA content (g/100 g sample)		
amino acids (AA)	dry blend	fortified puffed rice	% AA retention
leucine	1.75 ± 0.08	1.70 ± 0.01	97.1 ± 3.8
isoleucine	0.97 ± 0.00	0.95 ± 0.01	97.1 ± 0.9
valine	1.07 ± 0.01	1.14 ± 0.08	106.7 ± 9.0
methionine	0.36 ± 0.03	0.35 ± 0.00	97.5 ± 8.2
cysteine	0.36 ± 0.02	0.35 ± 0.01	97.4 ± 4.2
phenylalanine	1.13 ± 0.04	1.10 ± 0.01	97.3 ± 2.9
tyrosine	0.81 ± 0.06	0.77 ± 0.05	94.8 ± 0.6
lysine	1.30 ± 0.04	1.29 ± 0.05	98.6 ± 0.9
threonine	0.88 ± 0.05	0.84 ± 0.02	96.1 ± 4.0
histidine	0.56 ± 0.02	0.54 ± 0.00	96.7 ± 3.3
arginine	1.69 ± 0.09	1.63 ± 0.04	97.0 ± 2.9
serine	1.05 ± 0.08	1.00 ± 0.03	95.4 ± 4.1
alanine	1.05 ± 0.07	1.02 ± 0.03	97.0 ± 3.7
glutamic acid	3.90 ± 0.25	3.78 ± 0.09	97.2 ± 3.9
aspartic acid	2.40 ± 0.10	2.35 ± 0.04	97.8 ± 2.4
glycine	0.98 ± 0.07	0.94 ± 0.04	97.0 ± 3.6
tryptophan	0.29 ± 0.01	0.29 ± 0.00	99.7 ± 2.5
total amino acids	21.81 ± 1.18	21.21 ± 0.65	97.3 ± 2.3
crude protein (%)[b]	22.20 ± 0.15	22.14 ± 0.08	

[a] Values are on dry weight basis with means ± standard deviations. [b] Determined by Dumas combustion method (no. 6.25).

drastically affect true digestibility and net protein utilization of the processed proteins.[9] However, in the SCFX process, all of the essential amino acids including lysine and sulfur-containing amino acids were retained at very high levels, indicating the retention of nutritional quality of protein and the potential of the SCFX process to incorporate high amounts of proteins and bioactives in extruded products.

Protein Nutrient Quality. The essential amino acid content of the fortified puffed rice and its raw ingredients are given in Table 7. The amino acid profile has been compared with the essential amino acid requirement of a 2−5 year old child. The joint FAO/WHO consultation recommended the amino acid requirement of the age group as a reference amino acid profile to evaluate protein quality, as they are the most nutritionally demanding age group after infants.[31] The lysine content is low in many cereal grains; therefore, diets based on cereal grains can cause lysine deficiency. Rice protein contains only 35 mg lysine/g protein, which is much lower than casein (85 mg/g protein) or the requirement of the FAO/WHO consultation reference protein (58 mg/g protein).[31]

Table 7. Amino Acid Composition of Fortified Puffed Rice[a] **as Compared with Rice Flour, SPC, Casein, and Reference Protein**[b]

	amino acid content (mg/g protein)				
essential amino acids	reference protein[b]	casein	rice flour	soy protein concn	fortified puffed rice
histidine	19	32	21	26	25 ± 0.4
isoleucine	28	54	46	47	44 ± 1.4
leucine	66	95	80	77	79 ± 1.4
lysine	58	85	36	65	60 ± 1.1
methionine + cystine	25	35	52	26	33 ± 0.3
phenylalanine + tyrosine	63	114	98	83	88 ± 1.3
threonine	34	42	35	37	40 ± 0.1
tryptophan	11	14	12	12	14 ± 0.3
valine	35	63	63	49	54 ± 2.1
amino acid score[c]	1.00	1.00	0.66	0.84	1.00

[a] Data from this study. [b] Reference protein from joint FAO/WHO/UNU consultation (1985) used as a requirement of 2−5 year old child.[31] [c] The amino acid score was calculated using lysine as the primary limiting amino acid in rice and methionine as the primary amino acid in soy protein.

By combining rice flour with 8% bran and 22.5% soy protein, the lysine content improved from 35 to 60 mg/protein. Similarly, the amount of other essential amino acid also increased: histidine from 21 to 26 mg/g protein, threonine from 35 to 40 mg/g protein, and tryptophan from 12 to 14 mg/g protein. As a result, the amino acid score of rice protein improved from 0.64 to 1.0, which is equal to the score of a complete protein, and the contents of all essential amino acids were higher than the requirement of the reference protein (Table 7). The daily lysine requirement for humans is 1.0−1.5 g,[32] and the fortified puffed rice produced by SCFX contained 1.3 g/100 products (Table 6). The balanced ratio of the essential amino acids can improve the overall nutritional quality of the fortified puffed rice with improved protein qualities such as biological value, protein efficiency ratio, and net protein utilization.[31,32] These results indicate that it is possible to leverage novel technologies judiciously to maximize nutrition impact.

In summary, $SC-CO_2$-assisted extrusion was an effective process to produce protein, fiber, and micronutrients fortified puffed rice with balanced nutritional profile. The fortified rice contained 21.5% protein, 8% dietary fiber, and micronutrients at their recommended daily value of 18 mg of iron, 10 mg of zinc, 4990 IU of vitamin A, and 70 mg of vitamin C in 100 g serving size. Lysine, the major limiting amino acid in cereal grains, was present at 60 mg/g protein in the fortified product, as compared to 35 mg/g protein in the control, and had a very high level of retention (98.6%) during the extrusion process. The soy protein fortification improved the protein amount in the final product from 6.1 to 21.5%, and the complementary nature of the soy and rice proteins allowed for the final product to serve as a complete protein source. The amino acid profile indicated that the products contained all essential amino acids at a level equal to the reference protein requirement of a 2−5 year old child. Because the SCFX process used mild temperature and shear conditions, a total of 58% of vitamin A and 76% of vitamin C was retained during the SCFX process, with 55% of vitamin A and 64% vitamin C, with respect to the unprocessed formulation, retained after oven drying to 5−8% moisture. This study demonstrated that the SCFX process can be used in the production of cereal-based products fortified with multiple macro- and micronutrients and therefore can be effectively used to address multinutrient deficiencies in targeted populations.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 607-255-7913. Fax: 607-255-7913. E-mail: ssr3@cornell.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Sean Schell for his technical assistance in operating the extruder, Dr. Diane DellaValle, USDA/ARS, for her assistance with mineral analysis, ADM and Fortitech for providing soy protein and micronutrient premix for this study, and Wenger Manufacturing, Inc. (Sabetha, KS), for providing the TX-52 magnum extruder used in this research.

■ REFERENCES

(1) Benoist, B. D.; McLean, E.; Egli, I.; Cogswell, M. Worldwide prevalence of anaemia. *WHO Global Database on Anaemia*; World Health Organization: Geneva, Switzerland, 2008; 1993–2005.

(2) Alavi, S.; Bugusu, B.; Cramer, G.; Dary, O.; Lee, T. C.; Martin, L.; McEntire, J.; Wailes, E. *Rice Fortification in Developing Countries: A Critical Review of the Technical and Economic Feasibility*; Agency for Educational Development: Washington, DC, **2008**; http://members.ift.org/IFT/Research/.

(3) Dexter, P. B. *Rice Fortification for Developing Countries*; Opportunities for Micronutrient Interventions/USAID: Arlington, VA, 1998.

(4) Kapanidis, A. N; Lee, T. C. Novel method for the production of color–compatible ferrous sulfate–fortified simulated rice through extrusion. *J. Agric. Food Chem.* **1996**, *44*, 522–525.

(5) Rizvi, S. S. H. *VitaRice: A Novel, Low-Cost, Partially Cooked, Nutritionally Fortified Rice*; Institute of Food Technologists Annual Meeting, June 24–28, Orlando, Book of Abstracts, No. 007-02, 2006.

(6) Liu, Y.; Miller, D. D.; Rizvi, S. S. H. *Nutrient Retention in Fortified and Cooked Extruded Rice*; Institute of Food Technologists Annual Meeting, June 28–July 01, New Orleans, Book of Abstracts, No. 178-29, 2008.

(7) Yoo, J.; Alavi, S.; Adhikari, K.; Haub, M. D.; Aberle, R. A.; Huber, G. Rice-shaped extruded kernels: Physical, sensory and nutritional properties. *Int. J. Food Prop.* **2011**, DOI: 10.1080/10942912.2010.495042.

(8) Mishra, A.; Mishra, H. N.; Srinivasa Rao, P. Preparation of rice analogues using extrusion technology. *Int. J. Food Sci. Technol.* **2012**, DOI: 10.1111/j.1365-2621.2012.03035.x.

(9) Camire, M. E.; Camire, A.; Krumhar, K. Chemical and nutritional changes in foods during extrusion. *Crit. Rev. Food Sci. Nutr.* **1990**, *29*, 35–57.

(10) Rizvi, S. S. H.; Mulvaney, S. J.; Sokhey, A. S. The combined application of supercritical fluid and extrusion technology. *Trends Food Sci. Technol.* **1995**, *6*, 232–240.

(11) Sokhey, A. S.; Rizvi, S. S. H.; Mulvaney, S. J. Application of supercritical fluid extrusion to cereal processing. *Cereal Foods World* **1996**, *41*, 29–34.

(12) Wang, L.; Ganjyal, G.; Jones, D.; Weller, C.; Hanna, M. Modeling of bubble growth dynamics and nonisothermal expansion in starch-based foams during extrusion. *Adv. Polym. Technol.* **2005**, *24*, 29–45.

(13) Rizvi, S. S. H.; Mulvaney, S. J. Extrusion Processing with Supercritical Fluids. U.S. Patent 5120559, 1992.

(14) Sauceau, M.; Fages, J.; Common, A.; Nikitine, C.; Rodier, E. New challenges in polymer foaming: A review of extrusion processes assisted by supercritical carbon dioxide. *Prog. Polym. Sci.* **2011**, *36*, 749–766.

(15) Jacobs, J. M.; Kemmere, M. F.; Keurentjes, J. T. F. Sustainable polymer foaming using high pressure carbon dioxide: A review on fundamentals, processes and applications. *Green Chem.* **2008**, *10*, 731.

(16) Alavi, S. H.; Gogoi, B. K.; Khan, M.; Bowman, B. J.; Rizvi, S. S. H. Structural Properties of Protein-Stabilized Starch-Based Supercritical Fluid Extrudates. *Food Res. Int.* **1999**, *32*, 107–118.

(17) Alavi, S.; Rizvi, S. S. H. Supercritical fluid extrusion—A novel method for producing microcellular structures in starch-based matrices. In *Novel Food Processing: Effects on Rheological and Functional Properties*; Ahmed, J., Ramaswamy, H. S., Kasapis, S., and Boye, J., Eds.; CRC Press: Boca Raton, FL, 2009.

(18) Hicsasmaz, Z.; Dogan, E.; Chu, C.; Rizvi, S. S. H. Leavened Dough Processing by Supercritical Fluid Extrusion (SCFX). *J. Agric. Food Chem.* **2003**, *51*, 6191–6197.

(19) Hsieh, F.; Grenus, K. M.; Hu, L.; Huff, H. E. Twin-screw extrusion of rice flour with salt and sugar. *Cereal Chem.* **1993**, *70*, 493–498.

(20) Texture Technologies. Quantify Brittleness and Crispiness. Available on http://128.121.92.221/brittle.htm, 2005.

(21) Cornford, S. J. *Volume and Crumb Firmness Measurements in Bread and Cake*; FMBRA Report No. 25; CCFRA: Chipping Campden, United Kingdom, 1969.

(22) *Official Methods of Analysis of AOAC International*, 18th ed.; AOAC International: Gaithersburg, MD, 2006.

(23) AACC International. *Approved Methods of the American Association of Cereal Chemists*, 10th ed.; Methods 44-31. The Association: St. Paul, MN, 2000.

(24) Juliano, B. O. Rice bran. In *Rice: Chemistry and Technology*; Juliano, B. O., Ed.; American Association of Cereal Chemistry: St. Paul, MN, 1985; pp 647–687.

(25) Björck, I.; Asp, N.-G. The effects of extrusion-cooking on nutritional value. A literature review. *J. Food Eng.* **1983**, *2*, 281–308.

(26) Riaz, M. N.; Asif, M.; Ali, R. Stability of vitamins during extrusion. *Crit. Rev. Food Sci. Nutr.* **2009**, *49*, 361–368.

(27) Killeit, U. Vitamin retention in extrusion cooking. *Food Chem.* **1994**, *49*, 149–155.

(28) Cheftel, J. C. Nutritional effects of extrusion cooking. *Food Chem.* **1986**, *20*, 263–283.

(29) Camire, M. E. Nutritional changes during extrusion cooking. In Advances in Food Extrusion Technology; Maskin, M., Altan, A., Eds.; CRC Press: Boca Raton, 2011; pp 87–99.

(30) Singh, S.; Gamlath, S.; Wakeling, L. Nutritional aspects of food extrusion. A review. *Int. J. Food Sci. Technol.* **2007**, *42*, 916–929.

(31) FAO/WHO/UNU. *Energy and Protein Requirements. Report of a Joint FAO/WHO/UNU Expert Consultation*; WHO Technical Report; Series No. 724; WHO: Geneva, Switzerland, 1985; pp 138–149.

(32) FAO. *Protein Quality Evaluation, Report of the Joint FAO/WHO Expert Consultation*; FAO Food and Nutrition Paper No. 51; FAO: Bethesda, MD, 1991; pp 44–51.