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Fractionation of Hairless Canary Seed (*Phalaris canariensis*) into Starch, Protein, and Oil

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Canary seed is an important specialty crop in Canada. The current market for this true cereal (i.e., belonging to the family Poaceae as wheat) is limited to feed for caged birds. However, canary seed holds a promise for many food and industrial applications based on its composition. Three wet milling procedures based on ethanol (E), water (W), and alkaline (A) extractions used in different order were investigated to determine extraction efficiency and purity of starch, protein, oil, and fiber separated from hairless canary seed, a variety developed for human consumption. Highest extraction efficiencies were obtained when canary seed was defatted with ethanol and then extracted with alkali and water (EAW process). Using this process, approximately 92% pure starch, 75% pure protein, and oil were recovered from canary seed groats. The highest purity of protein, however, was obtained when canary seed was fractionated by the EWA process, that is, defatted and then extracted with water followed by alkali. Fiber component separated prior to alkaline extraction contained high amounts of nonfiber components as indicated by its yield. The EAW extraction process seems to be more promising in canary seed fractionation based on recovery and purity of components.

KEYWORDS: Canary seed components; crop fractionation; alkaline extraction; wet milling; component recovery

INTRODUCTION

Canary seed is one of the top four specialty crops grown in Western Canada with over 400 000 acres planted in this region (*1*). Currently this crop is solely used as food for caged and wild birds. However the unique composition and characteristics of canary seed make it a promising cereal for food and industrial uses (*2*). In the past, canary seed was not seen as a viable cereal for human consumption due to the harmful effects associated with the siliceous hairs that cover the hull of the seed. These hairs are highly irritating when they come in contact with human skin or lungs and have been linked to esophageal cancer (*3*, *4*). In 1997 CDC Maria was registered in Canada as the first hairless cultivar eliminating the potential health risk associated with hairy varieties (*5*). The variety was developed based on mutagenesis and traditional breeding by which a totally hairless variety was developed. Removing the damaging hairs rediscovered canary seed as a potential food crop and industrial crop for fractionation industry.

The main components in hairless canary seed are starch, protein, oil, and fiber (*2*). The groat of hairy canary seed contained approximately 60% starch, 20% protein, 7% total dietary fiber, and 8% crude fat (*6*). This cereal was also found to contain phytochemicals including phytates, phenols, tannins, and enzyme inhibitors (*2*). Fractionation of corn and small cereal grains has a long history and their components of starch, protein, and oil have been utilized in many food and nonfood applications. For instance, starch extraction from wheat was described as early as 234−249 B.C. by Marcus Porcius Cato (*7*). Today, cereals largely composed of corn and wheat grains are commonly used as sources of protein, starch, and oil, as well as other functional ingredients. Wheat gluten is often used as a protein supplement while corn is an important source of starch and oil (*7*).

It has been suggested that canary seed starch may be suited to the cosmetic industry due to the small and uniform size of canary seed starch granules compared with wheat starch (*8*). In addition, canary seed starch was found to differ from wheat and corn starches having lower content of amylose and higher peak viscosity and forming rigid and highly stable gel when cooked or frozen (*9*). Starches have also been used in textiles, adhesives, paper, food, and pharmaceutical industries as thickeners, colloidal stabilizers, and gelling agents (*10*, *11*).

Compared to the common cereals, canary seed contained high levels of protein at about 21% (*6*). Albumins and globulins in canary seed proteins were found at levels below that found in wheat (13.1% in canary seed versus 23.6% in wheat), while prolamines, which make up approximately 45.5% of the total protein, are more abundant in canary seed (*2*, *6*). This may reflect low levels of physiological proteins such as enzymes and enzyme inhibitors in canary seed. The amino acid composition of canary seed also highlights its unique protein composition and structure. Canary seed proteins were found to possess high concentrations

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of cystine, tryptophan, phenylalanine, and arginine and low levels of lysine and proline (*6*). In soybean protein, low lysine coupled with high arginine at ratio 0.9 lysine-to-arginine was found to exhibit hypocholesterolemic effects (*12*). The cholesterol-lowering effect of soy protein was also reported by Terpstra et al. (*13*). The lysine-to-arginine ratio in canary seed protein is much lower (0.2) compared with soy protein, which suggests that a hypocholesterolemic effect might be provided by canary seed proteins. In addition, canary seed proteins are exceptionally high in tryptophan (2.8 g/100 g protein), which would be an excellent supplement or blending protein for other cereals (e.g., wheat at 1.1 g/100 g protein) or animal proteins (e.g., casein at 1.0 g/100 g protein) that barely meet the FAO pattern of 1.1 g/100 g protein (*2*).

Fiber is extracted from some cereals due to its purported health benefits (*14*). For instance, dietary fiber intake from whole foods or supplements may improve serum lipid levels, reduce indicators of inflammation and lower blood pressure (*15*). Abdel-Aal and others (*6*) found that canary seed groats contain between 5.5 and 6.5% total dietary fiber, which is lower than most common cereals. Total dietary fiber content ranged from 11 to 25% in oats, 13−21% in wheat and 16−27% in barley (*14*, *16*). These data suggest that canary seed is not a good source of fiber.

While many food oils are extracted from oilseeds such as canola and soybean, cereal crops such as corn with oil content of approximately 4−5% are also common sources (*17*, *18*). Canary seed contained about 9% oil with fatty acid composition of 55% linoleic, 29% oleic, 11% palmitic, 2.5% linolenic, and 1% stearic (*6*). The oil is highly unsaturated suggesting a potential for rapid rancidity. However, the relatively low polyunsaturated fatty acid levels and the presence of antioxidant components in canary seed help delay rancidity during storage (*6*).

Alkaline extraction has been found to be an efficient method for the extraction of starch and protein from wheat (*19*, *20*), rice, (*21*) and canola or rapeseed (*22*). The objective of the present research was to develop an effective method for fractionating canary seed into starch, protein, fiber, and oil for food and nonfood uses. Three extraction procedures based on wet-milling fractionation, named EWA extraction process, EWWA extraction process, and EAW extraction process, were investigated to optimize component extraction efficiency and component yield. The extraction efficiency and components purity for each extraction process were evaluated. The extraction procedures were designed to study effects of fiber removal on the extraction efficiency of starch and protein, the main components in canary seed (about 80% of the groat). Previous studies have shown that canary seed protein and starch possess unique characteristics and hold a promise for food and cosmetic industries (*2*, *6*, *8*, *9*).

MATERIALS AND METHODS

Canary Seed Grains. The hairless canary seed (*Phalaris canariensis* L.) cultivar CDC Maria was obtained from the Crop Development Centre at the University of Saskatchewan (Saskatoon, Saskatchewan). Canary seed grains were dehulled using an abrasive dehuller. A cone-type dehuller that was designed and constructed by the department of Agricultural and Bioresource Engineering at the University of Saskatchewan was used to separate the hulls and to produce dehulled canary seed or canary seed groats. Details about the dehuller device and its efficiency are previously reported (*2*). Hulls were removed using air-aspiration to produce hull-free canary seed groats. The groats were then ground on a Udy Cyclone sample mill (UDY Co., Fort Collins, CO) with a 1 μm screen.

Extraction Procedures. Three methods of wet-milling fractionation based on extraction using ethanol, water, and alkaline were investigated. The fractionation methods were designed to separate canary seed components, e.g. oil, protein, starch, and fiber in different order through the modification of the sequence of the solvents. This allowed studying effects of fiber removal on the extraction efficiency of starch and protein from canary seed. Ethanol, an environmentally friendly solvent, is used in oil removal to avoid the health hazards of hexane and petroleum ether. **Figure 1** depicts the ethanol, water, and alkali (EWA) wet-milling extraction process. Using this method, canary seed components were extracted in the following order: oil, course fiber, protein, fine fiber, then starch. In this process, the ethanol extraction phase involved homogenizing 200 g of canary seed groat and 200 mL of ethanol in a Waring blender for 2 min at 15 000 rpm. Then the ethanol solubles were separated through centrifugation at 2800*g* for 15 min. The ethanol extraction was repeated three times and canary seed oil was recovered from the ethanol solubles in the supernatant (**Figure 1**). In the next step, water extraction was performed on the pellet. The sample and 2.25 L of water were blended with a homogenizer at 5000 rpm for 1 min, and then sieved through a 210 μm sieve three times. Coarse fiber was retained in the filter while fine solids and water passed through. Finally, alkaline extraction was carried out to separate protein from fine fiber and starch. The water and fine solids were mixed with 0.05N NaOH in a 1:10 ratio and stirred for an hour. The protein fraction was separated by centrifugation at 2800*g* for 15 min then precipitated from the supernatant by adjusting the pH to 4.0 using 4 N HCl. The centrifugal sediment (mainly starch) was scraped off to remove residual protein; then it was sieved through 70 and 52 μm sieves to separate fine fiber that was retained on the sieves. The starch in the pass through solids was recovered by centrifugation at 2800*g* for 15 min. The starch was then washed twice with 95% ethanol then dried with acetone.

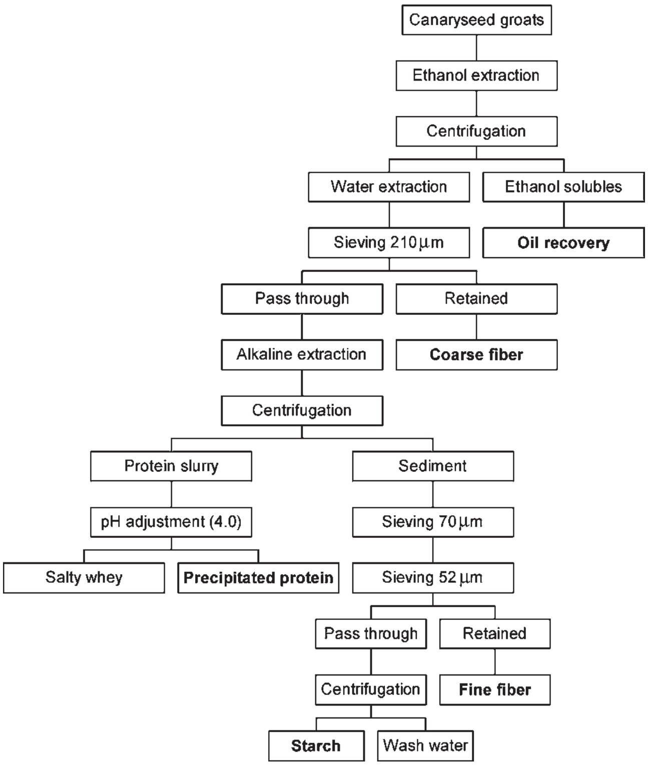

Figure 1. Fractionation of canary seed components by wet milling using EWA sequence extraction process.

The other two methods use the same chemical and mechanical conditions; however the order of solvents was altered. Ethanol, water, water, alkali (EWWA) follows a similar procedure to EWA process, but the alkaline extraction occurs at a later stage of the wet-milling process (**Figure 2**). The delay in alkaline extraction has resulted in changes in the order of component separation. In this method, oil separation is followed by coarse fiber, fine fiber, protein, then starch. This modification allowed the removal of coarse and fine fiber before extracting protein and starch. This order of component fractionation was also performed to study the impact of coarse and fine fiber removal on the extraction efficiency of protein and starch, the main components in canary seed. Again, ethanol was homogenized with canary seed and the oil was extracted through centrifugation. Then the water was blended in with the pellet for water extraction. The slurry was sieved through a 210 μm sieve. The coarse fiber was

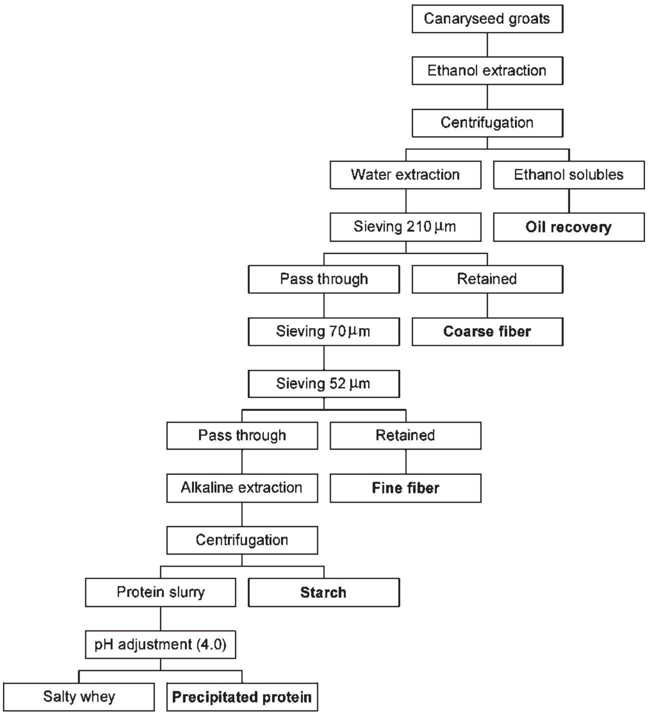

Figure 2. Fractionation of canary seed components by wet milling using EWWA sequence extraction process.

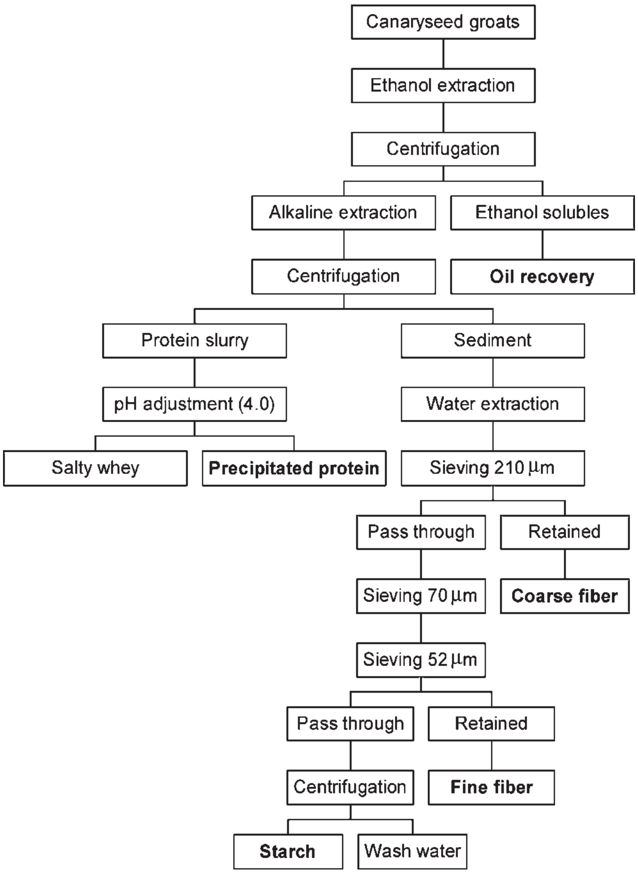

Figure 3. Fractionation of canary seed components by wet milling using EAW sequence extraction process.

retained, the material which passed through was then sieved through 70 and 52 μm filters, and the fine fiber was retained. After sieving, alkaline extraction using NaOH was applied to separate protein from starch as above.

The third method is the traditional alkaline extraction procedure in which, after removal of oil, the materials were extracted with alkaline solution. This method, the ethanol, alkaline, water (EAW), extracts oil, protein, and coarse fiber, fine fiber, and finally starch (**Figure 3**). As with the other two methods, ethanol was used to extract oil from the groats. Then protein was extracted from the sediment through alkaline extraction and centrifugation. The supernatant then went through water extraction and sieving to separate course fiber, fine fiber, and starch.

Extraction experiments were carried out at ambient temperature ($\sim$22 °C) in three replicates. Extraction efficiency was evaluated based on the total yield and purity of each separated component. Extraction efficiency was calculated based on crude isolate using the following equation: % extraction efficiency = total yield of a given component (g)/% of component in groat × 100. It was also calculated based on pure isolate as follows: % extraction efficiency = total yield of a given component (g) × % of component in isolate/% of component in groat × 100.

Chemical Analysis. Groat samples were ground and analyzed according to the AACC procedures (*23*) for moisture (Method 44-15A) and crude protein (Method 46-11A). The nitrogen-to-protein conversion factor was 5.7. In this study, the factor of 6.25 was not used because it is overestimated as it was reported that the nitrogen-to-protein conversion factor for cereals ranged from 5.61 to 5.93 and the factor of 5.7 is recommended (*24*). Starch was measured by the AACC approved method 76-13 (*25*). This method involves solublizing and partially hydrolyzing starch and starch containing materials into dextrans using fungal α-amylase. Dextrans were then completely broken down to glucose with amyloglucosidase and measured using the glucose oxidase/peroxidase reagent. Glucose was measure on a YSI Model 27 industrial analyzer (Yellow Springs Instrument Co., Yellow Springs, OH). Total dietary fiber was quantified by the AACC enzymatic gravimetric procedure (Method 32-21) (*23*). Oil was determined by gravimetric measurement after solvent evaporation according to the AACC procedure Method 30-20 (*23*). Total ash or minerals content was determined by dry ashing as outlined in the AACC procedure Method 80-03 (*23*).

Statistical Analysis. The data were subjected to analysis of variance to determine differences between extraction procedures on the basis of component extraction efficiency using Minitab software (version 12, Minitab inc., State College, PA). The data were reported as means of three (extraction experiments) or six (chemical analysis) replicates ± standard deviation (SD).

RESULTS AND DISCUSSION

The wet milling process is commonly used for the fractionation of a variety of cereal grains such as corn and wheat. Corn wet milling is used extensively to produce corn starch and corn oil (*26*). It is also used to isolate wheat gluten and starch. Wet milling involves physical and chemical alteration of seed components such as starch and protein and cell wall materials that leads to the dissociation of cell contents. Consequently, seed components such as fiber, oil, protein, and starch can be separated and recovered. In addition, alkaline extraction has been found to be efficient in separating starch and protein from cereal grains (*19−21*). Several reports have shown the potential of canary seed as a fractionation crop based on its unique composition of protein, starch, and oil (*2*, *6*, *9*). Data in **Table 1** show that canary seed on average has about 60% starch, 20% protein, and 8% oil. Canary seed is a true cereal belonging to the same family Poaceae as other cereals and has a unique composition of starch, protein, and oil and each component possesses unique characteristics and composition (*2*). For example, canary seed starch contains small uniform starch granules (*9*), canary seed protein is exceptionally high in tryptophan (*6*), and canary seed oil has high ratio of unsaturated to saturated fat (*6*). These compositions and characteristics make canary seed a potential crop for fractionation industry. The characteristics of canary seed starch show potential in cosmetic applications and protein could be used as a supplement in dairy products. Canary seed oil has a high ratio of unsaturated to saturated fat being 7.5 compared with 5.0 for wheat oil (*6*). It also contains higher oleic acid at about 29 versus

Table 1. Content of Main Components in Hairy Canary Seed Groats (Dry Basis, $n = 6$)

component	range	mean	standard deviation
starch	56.9−64.4	60.0	±2.6
protein	15.6−25.6	20.3	±3.2
total dietary fiber	5.5−8.3	6.6	±1.0
oil	7.6−8.9	8.3	±0.6
ash	1.9−2.1	2.0	±0.1

17% in wheat oil and lower polyunsaturated fatty acids (linoleic and linolenic) averaging 58 versus 67% in wheat oil (*6*). Such characteristics in addition to the presence of antioxidants would make canary seed oil a healthy choice.

In the present study, we investigated three procedures based on wet milling extraction to separate canary seed components, oil, starch, protein and fiber. Approximately 57% of the canary seed groat is comprised of starch on as is basis (**Table 1**). Of the three extraction methods, canary seed starch yield was the lowest with the EWWA extraction process (**Table 2**). The method produced 46.7 g starch per 100 g groat compared to 56.4 and 54.6 g per 100 g sample for the EAW and EWA extraction processes, respectively. All the three methods produced starch of high purity with little containments as indicated by starch concentration being about 93−95% (**Table 2**). The EWWA extraction process had starch fraction with about 5.3% nonstarch components compared with 6.7 and 7.1% for the other two processes. In general, the starch extraction efficiency calculated based on crude isolate was high for the three extraction methods ranging from 82 to 99% (**Table 2**). But the extraction efficiency of EAW and EWA was significantly greater than the EWWA process. Similar trend was obtained when the extraction efficiency was calculated based on pure isolate. There were significant differences between the EAW (92%) and EWA (88%) extraction processes compared with the EWWA process (78%). The extraction percentage is higher than the 60.2% obtained from wet milling of sorghum or 79% obtained from wet milling of buckwheat groat (*27*−*29*). Because of the small, uniform size of canary seed starch granules, extracting starch from this cereal has potential industrial and food applications including cosmetics as a gelling ingredient in skin lotions (*8*, *9*).

After starch, the next most abundant component in the canary seed kernel is protein. Canary seed contained about 20% protein higher than other cereal grains (**Table 1**). Once again the EAW and EWA extraction processes were both over 25% more efficient in extracting protein from canary seed than the EWWA extraction method (**Table 2**). These methods had extraction yields 24.1−24.5 g protein per 100 g groat compared with 17.3 g/100 g for EWWA method. The purity of the protein fraction was the highest for the EWA method, followed closely by EAW with the EWWA process had the lowest purity. Significant differences in extraction efficiency were observed between the extraction processes. The extraction efficiency of EAW method was 95.8 and 75.1% based on crude and pure protein, respectively, as compared with 67.4 and 51.0% for the EWWA process (**Table 2**). The extraction efficiency fell within or above the range reported in the literature for similar extraction methods using legumes. Bergthaller et al. (*30*) reported a 50% extraction efficiency using wet-milling separation for pea, whereas Wiege et al. (*31*) achieved approximately 90% efficiency by aqueous extraction followed by centrifugation. Protein isolated from canary seed groat may have a potential as supplementary or blending protein due to its high tryptophan content (*6*) and/or a health promoter due to its proposed hypocholesterolemic effects (*12*, *13*). Canary seed protein would be a good supplement in dairy products due to its high content of tryptophan (essential amino acid) as well as its possible lowering cholesterol effect.

Table 2. Content, Yield, and Extraction Efficiency of Canary Seed Starch, Protein, Fiber, and Oil Separated by Sequential Wet-Milling Extraction Processes (Dry Basis)[a]

			extraction efficiency (%)	
extraction process	concentration (%)	extraction yield (g/100 g)[b]	based on crude isolate[c]	based on pure isolate[d]
		Starch Isolate		
EWA	92.9 ± 2.1a	54.6 ± 2.0a	95.4a	87.9a
EWWA	94.7 ± 1.9a	46.7 ± 1.6b	82.0b	78.4b
EAW	93.3 ± 1.7a	56.4 ± 1.7a	99.1a	91.9a
		Protein Isolate		
EWA	82.7 ± 1.7a	24.1 ± 1.1a	93.6a	76.8a
EWWA	74.9 ± 2.6b	17.3 ± 0.7b	67.4b	51.0b
EAW	78.9 ± 2.4ab	24.5 ± 0.9a	95.8a	75.1a
		Fiber Isolate		
EWA	36.3 ± 1.4b	8.4 ± 0.3b	121.2b	91.3b
EWWA	31.0 ± 1.1c	18.1 ± 0.5a	264.2a	169.6a
EAW	59.6 ± 1.8a	5.6 ± 0.2c	81.9b	51.7c
		Oil		
all methods	100	5.7 ± 0.3	75.2	75.2

[a] For each isolated component means in a column with the same lower-case letter are not significantly different at $p < 5\%$. A stands for alkali, E stands for ethanol, and W stands for water. [b] g per 100 g canary seed groats. [c] (g total yield/% component in groats) × 100. [d] (g total yield × % component in isolate/% component in groats) × 100.

Compared to starch and protein, canary seed groat had a small amount of fiber averaging 6.6% (**Table 1**). The three extraction methods produced highly contaminated fiber isolate in which total dietary fiber content ranged from 31.0 to 59.6%, but still there were significant differences between the three extraction procedures in terms of fiber content, yield, and extraction efficiency (**Table 2**). The removal of protein and starch prior to fiber in the EAW was effective in obtaining relatively higher fiber-rich fraction, while separating fiber at early stage of the extraction process was not effective to obtain a fiber-rich fraction. Because of the high level of contamination in fiber fraction with starch and protein, the extraction efficiency values were greater than 100% in particular for the EWWA extraction method. The obtained fiber fraction would require further refining to obtain fiber-rich isolate.

Canary seed groat used in this study contained 8.3% oil (**Table 1**). Ethanol was found to be a sufficient solvent to extract oil from canary seed. Oil extraction was carried out similarly in the three methods and thus the data is presented on average. The ethanol extraction step was repeated thrice to extract as much of the oil from the groat. This way the extraction method achieved slightly over 75% extraction efficiency (**Table 2**). The extraction yield averaged about 5.7 g oil per 100 g of groat. Because of the higher oil content in canary seed, its extraction yield was higher than the 3.3 or 4.2 g/100 g obtained from corn at a solvent to solids ratio of 4 mL/g corn, an ethanol concentration of 100%, 30 min extraction time, and temperature of 50 °C either through a single batch extraction or three-stage extraction, respectively (*18*). But the extraction efficiency values were similar to those reported in the literature for other oilseeds. Dong and Walker (*17*) reported an extraction efficiency of 63% for canola while Kwiatkowski and Cheryan (*18*) and Sineiro et al. (*32*) reported 42 to 70% extraction efficiency for corn and sunflower oilseed, respectively. Canary seed with oil content of 8.3% is similar to oat groat that contains approximately 8% oil (*33*). Other cereals such as barley and wheat are low in oil content (2%), while the wheat germ contains about 11% and the wheat bran contains 2−3% (*34*, *35*). Canary seed groat could be considered a good source of edible oil based on the oil content and composition. In these extraction processes, oil would be a byproduct for the fractionation of starch and protein, the main components in canary seed. The oil has to be extracted either from the ground groat or the bran fraction as the germ of canary seed is very tiny and hard to be separated from the small canary seed groat (length 4.0−5.1 mm and width 1.5−2.0 mm) (*6*), as it has been done in the corn wet milling industry.

The sequence of separating components affects the quality of components separated from the groat. Since starch was always extracted last with all three methods, the order was less important to affect its extraction efficiency and purity. Protein and fiber were influenced most by the type of extraction process. For instance, extracting protein before fine fiber (e.g., EWA and EAW methods) resulted in a greater recovery with slightly less purity of protein than extracting fine fiber before protein (EWWA method). Contrary to this, the purity of fiber was higher when protein was extracted before fine fiber (EWA or EAW method). Thus the EWWA extraction method produced a greater contamination of fiber with proteins.

In general, we were able to fractionate canary seed starch, protein, and oil at realistic yield and recovery using a wet-milling process. The three extraction methods investigated were based on ethanol, water, and alkaline solution used in various sequences primarily to separate fiber before or after protein and starch and to study the impact of fiber separation on purity of starch and protein components. The extraction efficiencies of starch and protein were comparable to that reported for other cereals. The starch, protein, and oil isolated from canary seed would provide an additional supply to the industry in particular food and cosmetic industries. More research is underway to evaluate functionality of these components in certain applications.

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