Total choline and choline-containing moieties of commercially available pulses

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Abstract. Estimating choline intake is challenging using the current United States Department of Agriculture (USDA) database. The objectives of this study were to quantify the choline-containing moieties of pulses that are commercially available in North America and to use this information to determine the contribution of pulses to choline intake. Commonly consumed pulses (n=32) were analyzed by hydrophilic interaction liquid chromatography-tandem mass spectrometry (HILIC LC-MS/MS) and compared to the USDA database. Cooking reduced the relative percent from free choline and increased the contribution of phosphatidylcholine to total choline for most pulses ($P<0.05$). Using the expanded database to estimate choline content of recipes using pulses as meat alternatives, resulted in a different estimation of choline content per serving (±30%), suggesting that the USDA database could underestimate or overestimate choline intake in individuals or populations that consume pulses.

Keywords: Free Choline, Phosphatidylcholine, Phosphocholine, Glycerophosphocholine, Beans, Peas

Introduction

Choline is needed in the body for a variety of critical processes including the synthesis of the neurotransmitter acetylcholine, lipid transport by lipoproteins and methyl group donation (via the choline metabolite, betaine) [1]. The Institute of Medicine (IOM) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes has set current dietary recommendation for choline as Adequate Intake (AI) values, 550 mg/d for men and 425 mg/d for women [2]. The recommendation increases during pregnancy and lactation to 450 mg/d and 550 mg/d, respectively [2]. There is emerging epidemiological data estimating dietary choline intake in populations worldwide including North America [3-7], Jamaica [8], the Netherlands [9], New Zealand [10] and Norway [11]. In the North American population, choline intake has been examined in a number of large cohorts including the Frammingham Offspring Study [5], the Atherosclerosis Risk in Communities (ARIC) Study [3] and the Multiethnic Cohort (MEC) Study [6]. All of these cohorts that have attempted to estimate choline intake report that intake is below the AI. Free (unesterified) choline, phosphocholine, glycerophosphocholine, phosphatidylcholine, lysophosphatidylcholine and sphingomyelin are the different choline-containing moieties forms that contribute to total dietary choline. In a rodent model, the forms of choline have been shown to differ in absorption and metabolism [12], suggesting that an understanding of the different forms of choline rather than only total choline in a diet is needed.

Prior to 2004, a particular challenge in estimating choline intake was the absence of food composition information. In 2004, the United States Department of Agriculture (USDA) released the Database for the Choline Content of Common Foods (Release 1) which was later updated in 2008 (Release 2) [13]. The second release of the USDA database included betaine and total choline and forms (free choline, glycerophosphocholine, phosphocholine, phosphatidylcholine and sphingomyelin) for 634 foods, grouped into 22 food categories. Although this database serves as a source of choline composition for a wide variety of foods, other commonly consumed foods that provide dietary choline are not contained in the database. Specifically, in the "Legume and Legume Products" food category there is currently only choline composition data available for seven bean samples and other pulse varieties consumed in North America are absent from this database. It has also been reported that the major choline-containing moieties content of vegetables can vary with cooking due to enzymatic activity [14] and there is limited data on the choline content after cooking. Using the available data in the USDA database, pulses contain a mean of 49 mg total choline per 100 g, with major choline-containing moieties being free choline and phosphatidylcholine. A serving (3/4 cup, 175 mg) according to Canada's Food Guide [15] from this food category would provide approximately 20% of the recommended daily intake.

The objectives of the study described in this report were to 1) quantify the choline-containing moieties and the total choline content of a variety of pulses that are commercially available in North America and 2) use the expanded compositional database to determine the potential contribution of pulses to dietary choline intake.

Materials and Methods

Materials

L-α-Phosphatidylcholine (from egg yolk, ≥99%), sphingomyelin (from egg yolk, >95%), choline chloride (>98%), choline-trimethyl-d_9 (Cho-d_9) chloride, phosphocholine chloride calcium salt tetrahydrate (Sigma grade), betaine hydrochloride (>99%) were purchased from Sigma (St. Louis, MO); 1,2-distearoyl-sn-glycero-3-phosphocholine-N,N,N-trimethyld_9 (PC-d_9), 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-methyl (16:0 monomethyl-PE, MMPE), and L-α-lysophosphatidylcholine (egg, chicken) were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL). Glycerophosphocholine was supplied by Bachem Americas Inc. (Torrance, CA). Phosphocholine-N, N, N-trimethyl-d_9 (Pcho-d_9) chloride calcium salt was purchased from C/D/N Isotopes Inc. (Quebec, Canada). HPLC-grade ammonium formate (≥99%) and formic acid were supplied by Sigma (St. Louis, MO). Acetonitrile and water were of LC/MS grade from Fisher Scientific Company (Ottawa, ON, Canada). All other solvents were of HPLC grade.

HILIC LC-MS/MS method

The content (mg/100 g food) of choline-related compounds including free choline, glycerophosphocholine, phosphocholine, phosphatidylcholine, lysophosphatidylcholine, sphingomyelin and betaine in samples were determined using the hydrophilic interaction liquid chromatography-tandem mass spectrometry (HILIC LC-MS/MS) previously described [16, 17]. Briefly, an Agilent 1200 series HPLC system coupled to a 3200 QTRAP mass spectrometer (AB SCIEX, Concord, ON, Canada) under turbospray positive mode was used to analyze standard and sample solutions. The separation was carried out on an Ascentis Express 150 mm×2.1 mm HILIC column, 2.7 µm particle size (Sigma, St. Louis, MO). The column temperature was controlled at 25℃. The mobile phase A was acetonitrile and B was 10 mM ammonium formate in water at pH 3.0, adjusted using formic acid. The gradient was as follows: 0–0.1 min, 8% B; 0.1–10 min, from 8% to 30% B; 10–17 min, 95% B; and then back to 8% B at 17.1 min for column re-equilibrium prior to the next injection. The flow rate of mobile phase was 400 µl/min for the period from 20 min to 27 min and 200 µl/min for all other periods. The injection volume was 2 µl and the cycle time was 30 min/injection. Nitrogen was used as curtain gas, nebulizing gas and drying gas. Several scan modes, including precursor ion scan (Pre), neutral loss scan (NL) and multiple reaction monitoring (MRM) were used in order to quantify the target compounds, as described elsewhere [16, 17]. A valve was programmed by the data system to divert the LC effluent to waste before and after the selected retention time window from 2.5 min to 19 min. All other instrumental parameters were the same as previously reported [16, 17].

Sample preparation and extractions of phospholipids from pulses

To provide a more comprehensive table of the choline values of 32 samples of pulses were selected. To determine if the choline content of a pulse varied with cooking, the pulses were analyzed after different preparation methods including raw, canned and boiled. The analysis of 3 pulse varieties (kidney, pinto and soybean) was included for comparative purposes as these pulses are already included in the 2008 USDA Database for the Choline Content of Common Foods. The choline content of an additional 17 pulse food items that were not included in the USDA database were analyzed. Dry pulses, canned pulses and cooked pulses were purchased from local supermarkets. Dry pulses were ground and about 12 g of each was freeze dried. Canned pulses were drained, ground and freeze-dried. For measurements on cooked pulses, either dry pulses or canned pulses were cooked following the directions on the package. Cooked pulses were drained, ground and freeze-dried. The extraction procedure was described previously [16, 17]. Briefly, 100 mg of freeze-dried sample was homogenized in 2 mL of extraction solvent (chloroform:methanol:water, 1:2:0.8, v/v/v) for 5 min using a Polytron PT 1300D homogenizer at 10,000 rpm (Kinematica AG, Switzerland), then centrifuged at 1106 RCF (relative centrifuge force) for 5 min. The supernatant was collected and the extraction procedure repeated twice. The combined supernatant was diluted with methanol to a final volume of 10 mL in a volumetric flask. Around 1mL of this solution was filtered using syringe filter into a 2-mL HPLC vial and stored at -20°C prior to analysis.

After analysis was completed, the pulses were assigned a nutrient database number which corresponds with the USDA Nutrient Database for Standard Reference. For foods with no corresponding food description in the USDA Nutrient Database (no nutrient database number), they were not assigned a specific number and identified only by descriptive name. This occurred for the lentil varieties that were analyzed (green, red and canned lentils) as the only nutrient composition available was for raw lentils and cooked (boiled) lentils, lentil variety is not specified in the USDA database.

The application of updated choline content values in a meal rich in pulses

To examine the application of the updated choline database, w examined the effect of substituting the choline values available in the USDA database and the values obtained in our analyses in a group of pulse-based recipes (a gift of Dr. Rhonda Bell, University of Alberta). These recipes are currently being used in an intervention trial examining the effects of regular pulse consumption on blood lipid profiles. One serving of each recipe provides 120g of pulses, either beans or peas. The recipes includes in the analysis were for vegetable soup and tortellini soup. The choline values available from our analysis for black beans and yellow pears were used in estimating the choline content of the bean-based vegetable soup and the pea-based vegetable soup, respectively. The choline content of canned navy beans was used to estimate the choline content of these foods based on the USDA database values because navy beans were felt to be the most comparable bean given available data. Choline values for pinto beans were available from both our analysis of choline and the USDA database and this was used as the pulse ingredient in the tortellini soup.

Statistical Analysis

All data are presented as means ± SD, unless otherwise indicated. Choline content from each choline-containing moiety was expressed in milligram per 100 grams of sample. A two-tailed paired *t*-test was used to compare changes in choline-containing moieites from cooking. A *P*-value of <0.05 was considered statistically significant for all analyses.

Results and discussion

Choline content of commercially available pulses in Alberta

A total of 32 samples of commonly consumed pulses were analyzed for choline and choline moiety content (**Table 1**). Included in the table is the USDA nutrient database number (if available), food description, moisture content (5%), choline content arising from each choline-containing moiety (mg) and total choline (mg) per 100 g of sample. Many health organizations recommend pulse consumption as part of a healthy diet [18] and regular pulse consumption has been associated with reduced risk of cardiovascular disease and risk factors of metabolic syndrome [19,20]. In rodent studies, pulses have been shown to exhibit hypolipidemic effects [21, 22] and a meta-analysis of clinical trials examining the effect of pulses on serum lipoproteins concluded that consumption of approximately 100 g of uncooked pulses/day resulted in lowering of serum cholesterol [19]. In addition to being associated with positive effects on risk factors of cardiovascular disease and metabolic syndrome, pulses may also be a major dietary source of choline when consumed as a meat alternative. A 100 g daily serving of pulses, shown to positively effect cardiovascular disease risk, provides 15% of the current daily choline recommendation. A serving of pulses provides more dietary choline compared to many other reported major dietary sources of choline reported (dairy and meat) [3, 6, 10], see **Figure 1**.

Comparison of the choline content between the raw and cooked form of pulses

To examine changes with cooking, the proportion of choline arising from each of the choline-containing moieties in raw and cooked preparation methods as a percentage of total choline is presented in **Table 2**. Absolute values of each choline-containing moiety are included in **Supplementary Table 1**. In order to directly compare raw and cooked pulses, the choline contents of raw pulses were adjusted for moisture content and determined on a dry weight basis. Cooking significantly reduced (*P<0.05)* the relative percentage water soluble free choline to total choline in each of the pulse varieties (-9.7 ± 2.4%), with the exception of soybeans and green lentils. Cooking of soybeans or green lentils resulted in small increases in the relative abundance of choline that is present as free choline by about 2% in each case. Correspondingly, the contribution of phosphatidylcholine to total choline significantly increased (*P<0.05)* with cooking pulses, with the exception of soybeans and green lentils. Cooking of soybeans and whole green lentils decreased the percentage of choline arising from phosphatidylcholine by approximately -12% and -4%, respectively (see Table 2). For all pulse varieties, the contribution of lysophosphatidylcholine to total choline increased by 1.3 ± 0.2% (*P<0.05)* with cooking but it still remains a minor component of the overall choline content. The present study demonstrated that the cooking of pulses and discarding the cooking water resulted in a loss of water soluble compounds such as free choline. This is consistent with the work on vegetables by Zeisel et al.,[14]. It remains unclear as to why anomalous results were obtained for soybeans and green lentils, for which cooking results in a small increase in the relative contribution of free choline to total choline content along with a corresponding decrease in the relative contribution of phosphatidylcholine to total choline content.

Application of choline content of pulses to a meal rich in pulses

To determine the value of the expanded database on pulses, the choline content of a meal containing pulses as the main protein source was used. We examined the difference in choline content that resulted in substitutions of the nutrient values for pulses between the USDA database and our analyzed pulses for two recipes (vegetable soup and tortellini soup). **Table 3** lists the name of the pulse-containing meal used in this study, the pulse in the meal and the estimated choline content for one serving of the recipe. The estimate includes the choline content of the pulses and other food ingredients (calculated using USDA database). For two recipes, the USDA database overestimated total choline content by 23% and 6%. For a recipe using the same pulse (NDB No 16043), the USDA database underestimated total choline content for one serving by 30%. These results suggest that when pulses are a large part of a meal or diet, the use of accurate food composition data should be used. Pulse consumption varies between regions *(23)* and in Western countries consumption is considered low with consumption less than 3.5 kg/ capita per year [24]. However, in other populations with high pulse consumption (Africa, South America and India) where intakes range from 5 kg/ capita to 40 kg/ capita per year [24, 25, 26] pulses could make a major contribution to choline intake and the expanded database would be valuable in estimating intake. This was illustrated by the analysis of the vegetarian (pulse and pea) meals from the clinical trial taking place at the University of Alberta. Using meals from a study that relies heavily on pulses (120g pulses per serving) we observe a difference in the estimation of total choline based on available choline content from the USDA database and our values. To our knowledge there have been no published studies examining choline intake in vegetarian or vegan populations.

Conclusions

Our research adds valuable choline composition data for 32 pulses. A common limitation of studies examining dietary choline intake is the lack of choline composition values available for certain foods specific to the population. This occurred in a population of women from New Zealand [10], a multiethnic population in the United States [6] and a population of pregnant women from Jamaica [8]. The USDA Database for the Choline Content of Common Foods includes 634 foods, and the expansion of the current choline composition database will allow researchers to more accurately estimate usual intake and work towards establishing EAR and RDA values for choline.

In conclusion, the generation of accurate and comprehensive food composition data is essential for estimating usual dietary intake in populations that consume pulses as meat alternatives. The expanded pulse database in this manuscript will be useful in future research and in positioning pulses as a source of dietary choline.

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ABBREVIATIONS USED

AI, Adequate Intake; ARIC, Atherosclerosis Risk in Communities; HILIC LC-MS/MS, hydrophilic interaction liquid chromatography-tandem mass spectrometry; IOM, Institute of Medicine; MEC, Multiethnic Cohort; MRM, multiple reaction monitoring; NL, neutral loss scan; Pre, Precursor ion scan; RCF, relative centrifuge force; USDA, United States Department of Agriculture

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Fig. 1 Comparison of total choline in one serving of selected food items and food categories. Food categories corresponding to the USDA Choline Content of Common Foods and one serving size corresponding to Canada's Food Guide, with the exception of eggs which is half a serving. Pulses including kidney, navy, black and soybeans, one serving equal to 175 g (3/4 cup); Poultry including chicken and turkey, one serving equal to 75 g (½ cup); Beef excluding beef liver, one serving equal to 75 g (½ cup); Grains and Pastas including rice, bulgur and buckwheat, egg noodles and spaghetti, one serving equal to 125 g (½ cup); Milk including skim, 1%, 2% , whole and chocolate varieties, one serving equal to 250 g (1 cup); Vegetable including many varieties, with one serving equal to 125 g (½ cup).

Table 1 Choline content of commercially available pulses

NDB No[a]	Description	Moisture	Free Cho	GPC	Pcho	Ptd Cho	Lyso PC	SM	Total Cho[b]
		%	mg choline moiety/ 100 g of food (wet weight)						
16316[c]	Beans, black, canned	68.8	14.8	1.8	0.5	14.9	0.9	0.0	32.9
16015[c]	Beans, black, canned, cooked	66.7	13.7	1.7	0.4	14.3	0.9	0.0	31.0
[e]	Beans, black, cooked	63.8	14.8	3.1	0.5	24.4	0.9	0.0	43.7
[e]	Beans, black, raw	11.1	59.1	8.5	1.5	58.6	1.6	0.0	129.3
16358[c]	Beans, chickpeas (garbanzo), canned	69.1	5.8	1.3	0.6	23.9	3.2	0.0	34.8
16057[c]	Beans, chickpeas (garbanzo), canned, cooked	65.8	6.4	1.5	0.3	25.1	2.4	0.0	35.7
[e]	Beans, chickpeas, (garbanzo), whole, cooked	61.4	7.2	2.9	2.9	35.8	1.9	0.0	50.7
[e]	Beans, chickpeas, (garbanzo), whole, raw	7.6	23.5	5.6	1.9	88.4	3.3	0.0	122.7
16034[d]	Beans, kidney, red, canned	72.8	12.1	1.2	0.4	13.4	2.4	0.0	29.5
16071[c]	Beans, lima, raw	6.6	45.9	1.5	7.5	65.7	2.0	0.0	122.6
16146[c]	Beans, pinto, canned	70.7	8.4	0.8	2.3	11.8	1.5	0.0	24.8
[e]	Beans, pinto, canned, cooked	74.2	4.1	0.2	0.7	11.0	1.6		17.6
16043[d]	Beans, pinto, cooked	60.1	18.1	3.6	0.6	23.3	1.4	0.0	47.0
16042[d]	Beans, pinto, raw	7.2	59.9	8.0	1.3	56.3	3.0	0.0	128.5
[e]	Beans, red kidney, canned, cooked	70.8	13.8	1.4	0.3	13.4	1.6	0.0	30.5
[e]	Beans, red kidney, cooked	65.2	11.4	2.7	1.1	20.8	1.2	0.0	37.2
[e]	Beans, red kidney, raw	7.5	59.7	10.3	2.2	52.5	1.7	0.0	126.4
16045[c]	Beans, small white, raw	12.3	61.5	7.2	2.6	57.7	2.7	0.0	131.7

Table 1 Choline content of commercially available pulses

NDB No[1]	Description	Moisture	Free Cho	GPC	Pcho	Ptd Cho	Lyso PC	SM	Total Cho[2]
		%	mg choline moiety/ 100 g of food (wet weight)						
[e]	Beans, white kidney, canned	-	18.3	2.3	0.0	14.7	3.0	0.0	38.3
[e]	Lentils, canned	-	2.8	1.6	2.2	19.3	2.2	0.0	28.1
[e]	Lentils, green, whole, cooked	71.0	6.0	1.5	1.1	24.7	1.6	0.0	34.9
[e]	Lentils, green, whole, raw	7.4	18.4	4.4	4.2	90.9	3.2	0.0	121.1
[e]	Lentils, red, split, cooked	69.5	2.8	0.8	0.6	24.7	1.3	0.0	30.2
[e]	Lentils, red, split, raw	7.6	20.0	4.3	4.9	87.5	2.7	0.0	119.4
11813[c]	Peas, green, canned,	81.6	5.1	1.9	1.5	24.3	6.5	0.0	39.3
11811[c]	Peas, green, canned, cooked	79.7	4.9	1.9	1.6	27.6	6.6	0.0	42.6
[e]	Peas, green, split, cooked	63.8	7.8	3.6	1.4	26.2	1.5	0.0	40.5
[e]	Peas, green, whole, raw	7.9	18.1	8.4	3.0	68.0	6.3	0.0	103.8
16086[c]	Peas, yellow, split, cooked	63.7	6.6	4.0	1.1	28.2	1.4	0.0	41.3
16085[c]	Peas, yellow, split, raw	7.8	30.1	8.2	2.8	86.1	3.0	0.0	130.2
16109[c]	Soybeans, cooked	50.8	38.5	4.5	2.3	31.8	2.8	0.0	79.9
16108[c]	Soybeans, raw	6.7	71.5	10.6	3.1	66.8	3.9	0.0	155.9

NDB no (nutrient database number); Free cho (free choline); GPC (glycerophosphocholine); Pcho (phosphocholine); PtdCho (phosphatidylcholine); lysoPC (lysophosphatidylcholine); SM (sphingomyelin); Total Cho (total choline)

[a] NDB number is a numerical code used in the USDA Nutrient Database for Standard Reference [b] Total choline refers to the sum of free choline, GPC, phosphocholine, PC, lysoPC and SM and does not include betaine

[c] Indicates a pulse that is not included in the 2008 USDA Database for Choline Content of Common Foods where the accompanying choline values have been added to the assigned nutrient database number

[d] Indicates a pulse that is included in the 2008 USDA Database for Choline Content of Common Foods where the accompanying choline values have been modified based on analysis

[e] There was no specific NDB number for this pulse and only has description of pulse analyzed.

Table 2 Contribution percentage from choline-containing moieties to total choline between 100 g of raw and cooked pulses included in our analysis

Pulse	Prep[a]	Moisture[b]	Free Cho	PtdCho	LysoPC	GPC	Pcho
		%	% contribution of choline to total choline in 100g of pulses				
Black bean	Raw	11.1	45.7	45.3	1.2	6.6	1.2
	Cooked	63.8	33.9	55.8	2.1	7.1	1.1
Chickpeas	Raw	7.6	19.2	72.0	2.7	4.6	1.5
	Cooked	61.4	14.2	70.6	3.7	5.7	5.7
Dark red kidney beans	Raw	7.5	47.2	41.5	1.3	8.1	1.7
	Cooked	65.2	30.6	55.9	3.2	7.3	3.0
Pinto beans	Raw	7.2	46.6	43.8	2.3	6.2	1.0
	Cooked	60.0	38.5	49.6	3.0	7.7	1.3
Soya beans	Raw	6.7	45.9	42.8	2.5	6.8	2.0
	Cooked	50.8	50.8	48.2	3.5	5.6	2.9
Green lentils, whole	Raw	7.4	15.2	75.1	2.6	3.6	3.5
	Cooked	71	17.2	70.8	4.6	4.3	3.2
Red lentils, split	Raw	7.6	16.8	73.3	2.3	3.6	4.1
	Cooked	69.5	9.3	81.8	4.3	2.6	2.0
Green peas, split	Raw	6.3	26.2	62.3	2.6	7.4	1.4
	Cooked	63.8	19.3	64.7	3.7	8.9	3.5
Yellow peas, split	Raw	7.8	23.1	66.1	2.3	6.3	2.2
	Cooked	63.7	16.0	68.3	3.4	9.7	2.7

Free cho (free choline); GPC (glycerophosphocholine); Pcho (phosphocholine); PtdCho (phosphatidylcholine); lysoPC (lysophosphatidylcholine); Total Cho (total choline)

[a] Prep refers to the preparation method used for each pulse variety. Cooked pulses were boiled prior to analysis of choline content.

[b] Choline content has been adjusted based on moisture content of 100g of sample.

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Table 3 Comparison of the choline content between the USDA Choline Database and the expanded database (Edmonton) of one serving of meat substitute recipe using pulses as the main protein source

Meal[a]	Pulse Used	Database	Bet	Free Cho	GPC	Pcho	Ptd cho	Lyso PC	SM	Total Cho
			mg	mg choline moiety per serving						
Vegetable Soup	Black Beans[b]	USDA	0.6	37.5	3.0	3.0	32.2	0.0	0.0	76.3
		Edmonton	0.5	28.7	2.8	3.5	23.0	1.1	0.0	59.0
Vegetable Soup	Yellow Peas[c]	USDA	0.6	37.5	3.0	3.0	32.2	0.0	0.0	75.7
		Edmonton	0.5	20.2	5.5	4.3	39.7	1.7	0.0	71.4
Tortellini Soup	Pinto Beans[d]	USDA	9.3	18.2	3.9	1.7	30.2	0.0	0.6	54.5
		Edmonton	9.1	28.5	6.6	2.3	31.4	1.7	0.6	71.0

Free cho (free choline); GPC (glycerophosphocholine); Pcho (phosphocholine); PtdCho (phosphatidylcholine); lysoPC (lysophosphatidylcholine); Total Cho (total choline)

[a] Each recipe used for the meal contains 120 g of beans or peas per serving

[b]For USDA database pulse used was beans, navy, mature seeds, cooked, boiled, without salt (NDB No. 16038) and Edmonton database used beans, black, canned, cooked (16015).

[c]For USDA database pulse used was beans, navy, mature seeds, cooked, boiled, without salt (NDB No. 16038) and Edmonton database used peas, yellow, split, cooked (16086).

[d] For USDA database and Edmonton database pulse used was beans, pinto, mature seeds, cooked, boiled, without salt (16043).

[*] Indicates significant difference in total choline from values estimated using expanded choline database (includes additional analysis of pulses).

Fig. 1