Published in final edited form as:
J Cereal Sci. 2007 ; 46(1): 93–95. doi:10.1016/j.jcs.2006.11.002.

The betaine and choline content of a whole wheat flour compared to other mill streams

Ryan Likes[a], **Ronald L. Madl**[a], **Steven H. Zeisel**[b], and **Stuart A.S. Craig**[c,*]

[a]Department of Grain Science & Industry, Kansas State University, Manhattan, KS 66506, USA

[b]Department of Nutrition, School of Public Health and School of Medicine, University of North Carolina at Chapel Hill, NC 27599, USA

[c]Danisco USA Inc., Elmsford, NY 10523, USA

Keywords

Betaine; Choline; Whole Wheat; Milling

1. Introduction

Diets that contain high levels of whole grains have been associated with a reduced risk of several chronic diseases (Marquart et al., 2002). A whole grain, as defined by AACC International, should consist of the bran, germ and endosperm in the same proportions as found in nature (AACC, 1999). Whole grains are an important source of dietary fiber, resistant starch, vitamins, minerals and other bioactive nutrients that can play a role in protecting against chronic disease (Slavin, 2003). One of these nutrients is betaine (also known as trimethyl glycine or glycine betaine), an osmolyte and methyl donor shown to protect internal organs and improve vascular risk factors (Craig, 2004). As an osmolyte, it helps protect a variety of cells from osmotic stress. Betaine also plays an important role in cell biochemistry, donating a methyl group to homocysteine to produce methionine and ultimately *S*-adenosyl-methionine. Betaine accumulates in many plants under stress from drought, high salinity or low temperature. Cereal plants with betaine accumulation include wheat, barley and rye; those with little or no accumulation include rice, millet and sorghum (Kishitani et al., 1994). It has been suggested that betaine may be a factor in whole grain protection against coronary artery disease (Liu et al., 2000; Vos, 2000). Another of these nutrients is choline. Choline and betaine are structurally very similar, and choline is largely oxidized to betaine in the body. Choline is also metabolized to acetylcholine and phosphatidylcholine, and is essential for the normal function of cells (Zeisel, 2006). An early study showed that betaine and choline are nutrients in whole wheat which vary depending on wheat variety (Waggle et al., 1967). In addition, this study determined that these nutrients are particularly rich in the germ and bran. Recently, a USDA database has been developed showing the betaine and choline content of foods (Howe et al., 2004; Zeisel et al., 2003). Wheat-based foods can be important sources of betaine, and high levels were again found in wheat germ and bran. Finally, this database has been used recently to show that a periconceptual diet high in betaine and choline is associated with a reduced risk of infant neural tube defects (Shaw et al., 2004). Another epidemiological study shows that intake of choline and betaine predicts plasma homocysteine concentrations (Cho et al., 2006). Further epidemiological studies will emerge that utilize this new database to investigate other chronic

*Corresponding author. Tel.: 1 800 255 6837x2520. *E-mail address:* stuart.craig@danisco.com (S.A.S. Craig).

diseases. The objective of this study was to produce seven mill streams of wheat flour using a pilot mill to determine the effect of milling conditions on betaine and choline content.

2. Materials and methods

The grain used was a Kansas Hard Red Winter Wheat (HRW) composite sample of several local wheat varieties from the Co Op in Manhattan, KS. It is representative of the HRW class that would be used for bread flour in most Kansas mills. All the test fractions were produced from this single composite at the Kansas State University Pilot Mill using double roller mill stands and Great Western box sifters. A Ross table top roller mill was used to grind the whole wheat sample. The roller mills are 254 mm in diameter and 610 mm long. The seven millstreams were:

1. Whole wheat—ground to the particle size of flour using a table top Ross roller mill.
2. First Middlings Bottom Flour (1 MB FL)—the first grinding pass in the reduction system of the mill. The grinding stock contains the least bran, so the flour is the “cleanest” (least bran content) from the mill.
3. Sixth Middlings Flour (6 M FL)—the last grinding pass in the milling system. The flour from this reduction pass is the “dirtiest” (most bran content) in the milling process.
4. Fifth Break Flour (5 BK FL)—the last grinding pass in the break system of the mill. The break system is at the beginning of the milling process. Break grinding is not meant to produce a lot of flour, but to roll open the wheat kernel and scrape the endosperm from the bran. Fifth break grinding stock and flour contain quite a lot of bran.
5. Germ—separated in the pilot mill from the Second Quality (2Q) grinding stock. It is the material held over a 1050 μm sieve. The germ is then purified by a germ shaker (sieve) with a 1000 μm opening size (~18 mesh). This germ sample does contain some bran.
6. Red Dog—held over a 102 μm sieve (140 mesh) in the Sixth Middlings Sifter. It contains endosperm with quite a lot of bran.
7. Bran and Shorts (Millfeed)—the byproduct stream of the pilot mill. It contains large and small bran particles with some endosperm and some small germ particles. This stream is typically sent to a feed mill to be used in animal rations.

Betaine and choline metabolites of the above seven samples were extracted (Bligh and Dyer, 1959) then separated, analyzed and quantified directly by liquid chromatography/electrospray ionization-isotope dilution mass spectrometry after the addition of internal standards labeled with stable isotopes (Koc et al., 2002). Internal labeled standards were used to correct for recovery. The mass spectrometer used was a Thermo LCQ (Finnigan MAT). Each sample was analyzed in duplicate and an average value calculated.

3. Results and discussion

Table 1 shows the results of betaine and choline analysis of these flour fractions. As expected, the germ and bran contained the greatest concentration of betaine and choline. The bran had slightly more betaine content than the germ. The germ had twice the choline content of the bran. Betaine is present in much larger quantities than choline. This is in agreement with recent analysis of wheat-based products (Howe et al., 2004;Zeisel et al., 2003), but not with earlier work (Waggle et al., 1967). This is possibly a reflection of the more accurate modern methodology. Amongst the choline fractions, free choline was the largest component and

phosphocholine was the smallest. The 1 MB FL flour had the lowest amount of betaine and choline. In fact, the betaine content of 1 MB FL (~70 mg/ 100 g) was less than 25% that of the whole wheat flour (~300 mg/100 g). This is similar to the loss of other nutrients such as folate (Waggle et al., 1967) which ultimately led to recommendations to fortify flour. The effect of milling on choline levels was less dramatic than the effect on betaine.

The USDA database shows that the betaine content of cereal-based foods varies from less than 10 mg to more than 1500 mg per 100 g food. The range for wheat-based foods is similar, however corn, rice and oat products appear to be much lower in betaine content. Wheat is likely to be the main dietary source of betaine, since other rich sources (>100 mg/100 g) are eaten in lower quantities (e.g. spinach, beets and shellfish). Table 2 (first column) shows the estimated annual wheat consumption in selected countries in 2000 using data from the FAO website (FAO, 2006). Table 2 also shows an estimate of the daily betaine intake calculated using the annual consumption of wheat from the first column and our data on betaine content of wheat from Table 1 (i.e. ~300 mg/100 g in whole wheat flour and ~70 mg/100 g in refined flour). For example, in the USA, consumption of wheat as solely whole wheat would lead to an intake of 699 mg/d betaine whereas consumption as solely refined wheat would lead to an intake of 163 mg/d betaine. Recent published data using the USDA database (Cho et al., 2006;Shaw et al., 2004) suggests that the mean betaine intake in the US is currently ~200 mg/d. This may be lower than desirable; however there is currently no estimate of adequate intake (AI) for betaine. The AI for choline is 550 mg/d for men, and 425 mg/d for women (IOM, 1998). It is interesting to note that two of the richest dietary sources of betaine, wheat and beets, are refined to flour and sugar, respectively. These important dietary carbohydrates are a large component of the diet and no longer contain much betaine.

4. Conclusions

There is growing interest in determining the contribution of whole grain bioactive components to health benefits. In many countries, refined flour is fortified with some of the nutrients lost during milling (e.g. B-vitamins and some minerals). However, there are many other nutrients that remain lost. Betaine and choline work in concert with some of the B-vitamins and zinc to provide methyl groups in the methionine cycle. Diets low in these methyl donors lead to elevated serum homocysteine levels, a risk factor for chronic disease (Craig, 2004; Stead et al., 2006; Zeisel, 2006). Intake of betaine will be significantly lower if diets contain foods made predominantly with refined wheat rather than whole wheat. The intake of choline will also be somewhat lower. Future recommendations regarding dietary supplementation, or flour fortification, may include more bioactive components like betaine and choline. However, further work is needed to better understand the variation in cereals due to species and growing conditions in the content of these components. This study further shows that processing of wheat has a significant effect on the delivery of dietary methyl groups—particularly betaine. This may be a factor in the health benefits of whole grain.

References

AACC. Definition of whole grain. 1999. http://www.aaccnet.org/definitions/wholegrain.asp

Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 1959;37:911–917. [PubMed: 13671378]

Cho E, Zeisel SH, Jacques P, Selhub J, Dougherty L, Colditz GA, Willett WC. Dietary choline and betaine assessed by food-frequency questionnaire in relation to plasma total homocysteine concentration in the Framingham Offspring Study. American Journal of Clinical Nutrition 2006;83:905–911. [PubMed: 16600945]

Craig SAS. Betaine in human nutrition. American Journal of Clinical Nutrition 2004;80:539–549. [PubMed: 15321791]

FAO. Wheat Crop Info. 2006. http://www.fao.org/ag/agp/agpc/doc/field/Wheat/data.htm

Howe, JC.; Williams, JR.; Holden, JM.; Zeisel, S.; Mar, M. USDA database for the choline content of common foods. US Department of Agriculture. 2004. http://www.nal.usda.gov/fnic/foodcomp/Data/Choline/Choline.html

IOM. Pantothenic Acid, Biotin and Choline. Vol. 1. National Academy Press; Washington, DC: 1998. Dietary Refererence Intakes for Folate, Thiamin, Riboflavin, Niacin, Vitamin B12.

Kishitani S, Watanabe K, Yasuda S, Arakawa K, Takabe T. Accumulation of glycinebetaine during cold acclimation and freezing tolerance in leaves of winter and spring barley plants. Plant Cell and Environment 1994;17:89–95.

Koc H, Mar MH, Ranasinghe A, Swenberg JA, Zeisel SH. Quantitation of choline and its metabolites in tissues and foods by liquid chromatography/electrospray ionization-isotope dilution mass spectrometry. Analytical Chemistry 2002;74:4734–4740. [PubMed: 12349977]

Liu S, Manson J, Hu F, Willett W. Reply to E Vos. American Journal of Clinical Nutrition 2000;71:1009. [PubMed: 10731513]

Marquart, L.; Slavin, JL.; Fulcher, RG. Whole-grain Foods in Health and Disease. American Association of Cereal Chemists; St. Paul, MN: 2002.

Shaw GM, Carmichael SL, Yang W, Selvin S, Schaffer DM. Periconceptional dietary intake of choline and betaine and neural tube defects in offspring. American Journal of Epidemiology 2004;160:102–109. [PubMed: 15234930]

Slavin J. Why whole grains are protective: biological mechanisms. Proceedings of the Nutrition Society 2003;62:129–134. [PubMed: 12740067]

Stead LM, Brosnan JT, Brosnan ME, Vance DE, Jacobs RL. Is it time to reevaluate methyl balance in humans? American Journal of Clinical Nutrition 2006;83:5–10. [PubMed: 16400042]

Vos E. Whole grains and coronary heart disease. American Journal of Clinical Nutrition 2000;71:1009. [PubMed: 10731513]

Waggle DH, Lambert MA, Miller GD, Farrel EP, Deyoe CW. Extensive analyses of flours and millfeeds made from nine different wheat mixes. II. Amino acids, minerals, vitamins, and gross energy. Cereal Chemistry 1967;44:48–60.

Zeisel SH. Choline: critical role during fetal development and dietary requirement in adults. Annual Reviews in Nutrition 2006:5.1–5.22.

Zeisel SH, Mar MH, Howe JC, Holden JM. Concentrations of choline-containing compounds and betaine in common foods. Journal of Nutrition 2003;133:1302–1307. [PubMed: 12730414]

J Cereal Sci. Author manuscript; available in PMC 2008 November 21.

Table 1

Betaine and choline content of flour mill streams

	Betaine[a]	Choline[b]	GPC[b]	PCho[b]	PtdCho[b]	SM[b]	Total choline[b]
Bran	1293.3	47.3	9.5	0.5	27.3	3.8	88.4
Germ	1163.5	114.9	26.9	1.0	22.2	3.4	168.4
6 MFL	360.7	14.8	8.1	0.7	32.4	6.0	62.0
Whole W.	291.2	14.4	3.8	0.2	5.5	3.4	27.3
5 BKFL	169.1	9.4	3.1	0	5.6	4.1	22.2
Red Dog	92.4	4.5	1.5	0.4	7.4	5.1	18.9
1 MBFL	71.8	3.6	1.3	0	7.1	5.0	17

Abbreviations (see text for more details): Bran, bran and shorts; Germ, germ; 6 MFL, sixth middlings flour; Whole W., whole wheat; 5 BKFL, fifth break flour; Red Dog, red dog; 1 MBFL, first middling bottom flour; GPC, glycerophosphocholine; PCho, phosphocholine; PtdCho, phosphatidylcholine; SM, sphingomyelin.

[a]Units: mg/100 g of flour.

[b]Units: mg choline moiety/100 g of flour.

Table 2

Estimate of betaine intake from consumption of whole wheat vs. refined wheat in various countries

	Annual wheat consumption (kg per capita) (FAO, 2006)	Estimated daily betaine consumption (mg) from whole wheat (containing 300 mg betaine/100 g flour)	Estimated daily betaine consumption (mg) from refined wheat (containing 70 mg betaine/100 g flour)
Algeria	189	1553	362
Italy	150	1233	288
Egypt	137	1126	263
Israel	131	1077	251
France	98	806	188
USA	85	699	163
China	78	641	150