

Analytical, Nutritional and Clinical Methods Section

Determination of nutritionally important starch fractions of some Turkish breads

A. A. Tas*, S.N. El

Engineering Faculty, Food Engineering Department, Ege University, 35100, Bornova, Izmir, Turkey

Received 17 May 1999; accepted 16 September 1999

Abstract

By using an in vitro technique that measures the rate and extent of starch digestion, the starch in a food can be classified as rapidly-digestible starch (RDS), slowly-digestible starch (SDS) and resistant starch (RS). The amount of these fractions in rye bread, wheat bran bread and corn flour bread were calculated from the experimentally determined values obtained by controlled enzymatic hydrolysis and measurement of released glucose by colorimetry, using a glucose oxidase kit. Free glucose and total starch analysis were also carried out to calculate rapidly-available glucose (RAG) content and starch digestibility rate index (SDRI) of breads. RDS content of the rye bread was higher than the wheat-bran bread and corn flour bread ($P < 0.05$). SDS contents of all breads were not significantly different from each other. Wheat bran bread was found to have a significant amount of RS (6.6%; on dry matter basis) when compared to the other breads ($P < 0.05$). A significant negative correlation ($r = -0.95$, $P < 0.05$) was also found between RS content and SDRI value of breads. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

For nutritional purposes, Englyst and Cummings (1993) classified the starch in foods as rapidly-digestible starch (RDS), slowly-digestible starch (SDS) and resistant starch (RS). Three major forms of RS in the diet have been identified as physically inaccessible starch (RS₁), resistant starch granules (RS₂) and retrograded starch (RS₃) (Englyst, Veenstra & Hudson, 1996; Englyst & Hudson, 1996; Asp, 1996).

Resistant starch is defined as the starch and starch degradation products not absorbed in the small intestine of healthy humans (Englyst & Cummings, 1993; Englyst, Kingman, Hudson & Cummings, 1996). Starch remaining unabsorbed and passed through the colon has been shown to have important physiological effects. Short chain fatty acid synthesis, bacterial activity, epithelial cell function and nitrogen metabolism are controlled by the carbohydrates entering the colon (Berggren, Björck, Margareta, Nyman & Eggum, 1995; Cummings, Beatty, Kingman, Bingham & Englyst, 1996; Edwards et al., 1996). Like dietary fibre, RS is

used as a substrate by the microorganisms in the colon resulting in the production of hydrogen, carbon dioxide, methane and short chain fatty acids (Cummings & Englyst, 1995; Gordon, Topp, Shi, Zallie & Jeffcoat, 1997; Kritchevsky, 1988; Martin, Duman & Champ, 1998).

The extent of RS formation in a food depends on a number of factors, including the botanical source of the starch as well as type of processing. Physical state (whole or ground), composition and moisture content of the food, pH, heating time and temperature, and number of heating and cooling cycles are the main factors reported to affect the rate of RS formation (Annison & Topping, 1994; Heaton, Marcus, Emmett & Bolton, 1988; Kingman & Englyst, 1994; Szczodrak & Pomeranz, 1992; Würsch, Vedovo & Koellreutter, 1986) There is a positive correlation between the amylose content of starches and the yield of RS that can be produced by autoclaving and cooling (Sievert & Pomeranz, 1989). Heat treatment usually decreases the RS₁ and RS₂ contents but causes an increase in RS₃ content of foods (Skrabanja & Kreft, 1998).

Bread constitutes the main part of one or more of the daily meals and cannot be easily replaced in contrast to other rapidly-digested base foods. The amount of RS

* Corresponding author.

delivered to the large bowel by a Western diet was recently estimated as 2.5–5 g, which was considerable (Holm & Björck, 1992). Estimated annual intake of bread in European countries ranges from 46 to 100 kg per person (Liljeberg, Akerberg & Björck, 1996). In Turkey, the annual intake of bread was reported to range from 180 to 210 kg per person (Ünal, 1991). Being a developing country, carbohydrates constitute the main part of the daily energy intake (55 to 60%) of people in Turkey, according to Turkish Recommended Daily Allowances, 5–6 slices of bread (250–300 g) are recommended to be consumed daily. Six to 11 servings of cereal group per day in Ideal Diet Pyramide are also supplied by bread consumption because bread is readily available, cheap and nutritious. In view of the quantitative importance in the Turkish people's diet, bread can be expected to be one of the major sources of RS.

A number of foods, such as peas, beans, potatoes, banana and cereals, have been shown to contain various amounts of RS (Goni, Garcia-Diz, Manas & Saura-Calixto, 1996; Englyst, Veenstra et al., 1996). Attempts to increase the RS content of bread products have been made by adding defined starch fractions to bread (Liljeberg et al., 1996; Unlu & Faller, 1998). Two new types of commercially-prepared RS (Novelose™ and Crystalean™) were introduced in 1994 to be used as new ingredients for the food industry (Alexander, 1995). There is no published information on the RS content of the foods consumed in Turkey. Being the most commonly consumed staple food, bread is an ideal material to investigate the RS content of the foods in the Turkish people's diet.

The purpose of the present study was to determine the nutritionally important starch content of three most commonly used breads in Turkey, namely rye bread, wheat bran bread and corn flour bread by using the indirect method of Englyst, Veenstra, et al. (1996). Rapidly-available glucose (RAG) content and starch digestibility rate index (SDRI) value for breads were also calculated.

2. Materials and methods

2.1. Materials

Enzymes (pepsin, pancreatin, invertase and amyloglucosidase), guar gum and reference samples were kindly provided by Hans N. Englyst. Glucose oxidase colorimetric kit (Boehringer glucose (GOD-PAP) test combination, cat. no: 676543) was purchased. Commercially prepared breads were purchased from a local supermarket in Izmir. The composition of the breads on a weight basis are shown in Table 1. Rye bread, wheat bran bread and corn flour bread were proofed at 28°C for 20–25 min and baked at 230°C for 30 min.

Table 1
The composition (on weight basis) of the breads studied

Bread type	Composition	Amount (%)
Rye bread	72% Extraction wheat flour	67
	60–65% Extraction rye flour	26
	Bread mixture ^a	7
Wheat bran bread	72% Extraction wheat flour	85
	Wheat bran	8
	Bread mixture ^a	7
Corn flour bread	72% Extraction wheat flour	71
	35% Extraction corn flour	23
	Bread mixture ^a	6

^a Bread mixture contains emulgator, enzyme, preservative and wheat germ.

2.2. Methods

Total starch, nutritionally important starch fractions (rapidly-digestible starch, slowly-digestible starch and resistant starch) and free glucose content of the breads were measured by the method of Englyst, Veenstra et al. (1996). Moisture analyses of the breads were carried out according to the method of the Turkish Standards Institution (TS 5000) (Türk Standartlar Enstitüsü, 1987). Nutritionally important starch fractions analysis of Englyst et al. (1996) is based on the controlled enzymatic hydrolysis of starch and measurement of the released glucose using a glucose oxidase kit. It is described in brief below.

Before analysing test samples, reference potato starch was carried through the procedure to check that the stroke speed of the shaking bath was appropriate. After obtaining target values for potato starch, analyses were repeated with other references (e.g. wheat flour and corn flakes).

Ten hours after the breads were baked, the analyses were started. The breads were passed through a domestic mincer and weighed (between 1000 and 2000 mg to the nearest 0.1 mg) into the polypropylene centrifuge tubes. Samples were incubated with pepsin at 37°C for 30 min for the removal of protein. After that, the samples were incubated with pancreatin, invertase and amyloglucosidase at 37°C and pH 5.2 in capped tubes immersed horizontally in a shaking bath. After 20 and 120 min incubation, 0.5 ml samples of hydrolysate were removed and placed in 66% ethanol to halt the reaction. The hydrolysates in the main sample tubes were kept in a boiling water bath for 30 min to gelatinize any granular starch. The hydrolysates were then chilled in ice water and retrograded starch was dispersed by using 7 M potassium hydroxide solution and shaking the tubes in an ice water bath for 30 min. A portion from this solution was placed in 0.5 M acetic acid, amyloglucosidase solution was added and the tubes kept at 70°C for 30 min. A separate sample of the bread was incubated with

invertase alone to correct for free sugars. The values obtained for glucose released after 20 and 120 min enzyme incubation, and after treatment with heat and alkali (corrected for free sugars) were used to calculate total, rapidly-digestible, slowly-digestible and resistant starch fractions.

2.3. Statistical analysis

All results were expressed as means \pm S.E.M. Data collected from this study were analysed by one-way analysis of variance and Duncan's procedure for multiple comparison to determine significant differences in nutritionally important starch fraction contents of the breads. A value of ($P < 0.05$) was considered significant.

3. Results and discussion

Total starch (TS) content of the breads ranged from 70 to 77% on a dry matter basis (Table 2). Rye bread and wheat bran bread were found to contain significant amounts of TS when compared to corn flour bread ($P < 0.05$). Nutritionally important starch fractions of the breads are shown in Fig. 1.

Rye bread, wheat bran bread and corn flour bread were found to contain 73.4, 68.0 and 65.9% RDS, respectively. RDS content of the rye bread was higher than wheat bran bread and corn flour bread ($P < 0.05$). Englyst, Kingman and Cummings (1992) reported that the breads made of wheat flour and whole wheat flour contained significant amounts (50–69%, dry matter basis) of RDS.

Englyst, Veenstra et al. (1996) analysed the nutritionally important starch fraction content of 39 starchy foods whose glycemic indices (GI) were obtained from the literature and reported a highly significant ($P < 0.001$) positive correlation between glycemic index and both RDS and RAG. Since RAG value relates to the food as eaten and includes both RDS and free glucose, it was reported to be a better indicator for blood glucose and insulin response than the SDRI (Englyst et al., 1992). SDRI is rapidly digestible starch expressed as percentage of the total starch in the food (Kingman &

Englyst, 1994). It reflects the rate of digestion but does not give any information about the actual amount of glucose likely to be rapidly available from the food (Englyst, Veenstra et al., 1996)

SDRI and RAG of the breads are given in Table 3. SDRI value of the breads ranged from 88.8 to 94.2. There was a slight difference between SDRI of rye bread and corn flour bread whereas wheat bran bread had a lower SDRI ($P < 0.05$). A significant negative correlation ($r = -0.95$, $P < 0.05$) was found between RS content and SDRI of the breads. The differences in RAG contents of the rye bread, wheat bran bread and corn flour bread were found to be statistically significant ($P < 0.05$) and varied between 73.5 and 81.7% (on dry matter basis). Englyst et al. (1992) reported that wheat bread and whole rye bread had RAG values of 77 and 62% (on dry matter basis), respectively.

SDS content of the breads ranged from 1.9 to 2.4%. Similarly, lower amounts of SDS have been shown in various types of bread (4–7% on dry matter basis) and breakfast cereals (2–4% on dry matter basis) where starch is in easily digestible form (Englyst et al., 1992).

When comparing all the breads, the highest resistant starch level (6.6% on dry matter basis) was detected in the wheat bran bread ($P < 0.05$). RS contents of rye bread and corn flour bread were found to be 2.7 and 2.2%, respectively. In a suggested classification made by Goni, Garcia-Diz, Manas and Saura-Calixto (1996a), breads are described as low RS content (containing 1–2.5% RS on dry matter basis) foods. Englyst and Hudson (1996) reported that RS contents of the wheat bread and whole wheat bread were 0.6 and 1.5% (on dry matter basis), respectively, whereas bread made of whole rye flour was found to have 3.2% RS. Liljeberg et al. (1996) reported that RS content of the bread containing whole rye flour and wheat flour (70:30) and baked at 230°C for 30 min was 2.7%.

The small amounts of RS in breads and breakfast cereals are mainly retrograded amylose (Englyst et al., 1992). In vivo experiments showed that retrograded amylose was the major undigested starch fraction in white wheat bread and autoclaved wheat starch (Björck & Siljeström, 1992). Heating the foods in a high moisture environment can result in an increase in the RS content by affecting the rate or retrogradation due to an increase in gelatinized starch content. The high RS content in bread made of white wheat flour and wheat kernels (20:80) was probably related to the repeated heating and cooling of the starch in the whole grains (boiling to soften the grains and baking) as well as to the higher moisture content in the dough (Holm and Björck, 1992). In this study, baking time and temperature of the breads remained the same but the amount of water added for each 100 units of dry matter were 38, 42 and 39 units for rye bread, wheat bran bread and corn flour bread, respectively. Addition of greater amounts

Table 2
Nutritionally important starch content of the breads (g/100 g dry matter)^{a,b,c}

Breads	TS	RDS	SDS	RS
Rye bread	77.4 \pm 3.8a	73.4 \pm 0.9a	1.9 \pm 0.1a	2.7 \pm 2.3a
Wheat bran bread	76.6 \pm 2.6a	68.0 \pm 1.3b	2.0 \pm 0.5a	6.6 \pm 1.5b
Corn flour bread	70.5 \pm 1.6b	65.9 \pm 1.0b	2.4 \pm 0.1a	2.2 \pm 0.7a

^a Means \pm S.E.M., $n = 6$.

^b Values not sharing the same letters are significantly different ($P < 0.05$).

^c TS, total starch; RDS, rapidly-digestible starch; SDS, slowly-digestible starch; RS, resistant starch.

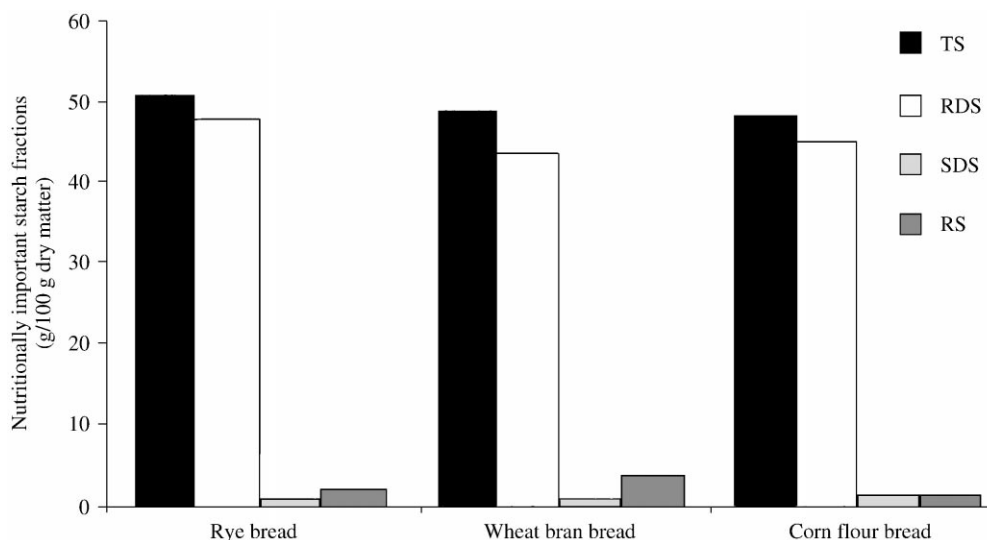


Fig. 1. Nutritively important starch fractions of the breads.

Table 3
Starch digestibility rate index (SDRI) and rapidly available glucose (RAG) content of the breads ^{a,b,c}

Breads	SDRI	RAG (g/100 g dry matter)
Rye bread	94.2 ± 1.6a	81.7 ± 1.1a
Wheat bran bread	88.8 ± 0.9b	76.2 ± 1.5b
Corn flour bread	93.4 ± 0.4a	73.5 ± 0.9c

^a Means ± S.E.M., *n* = 6.

^b Values not sharing the same letters are significantly different (*P* < 0.05).

^c RS, resistant starch; SDRI, starch digestibility rate index; RAG rapidly-available glucose.

of water to wheat bran bread mixture can affect the degree of gelatinization and therefore RS formation.

Oat bran bread containing white wheat flour, oat bran and gluten (50:45:5) and baked at 210°C for 22 min was reported to have a RS content of 0.6 g/100 g dry matter (Holm & Björck, 1992). Goni et al. (1996) found that a breakfast cereal containing high proportion of bran had a negligible RS content (less than or equal to 1% dry matter). In a study of Englyst, Veenstra et al. (1996), oat bran and all bran breakfast cereals were found to contain 1.0 and 1.1 g RS/100 g as eaten, respectively. It was reported that the content of the bread products may be affected by the recipe and baking conditions (Holm & Björck, 1992). The difference in RS content of the wheat bran bread studied and other breads containing bran may be due to difference in materials and processing parameters used.

Dietary fibre has been suggested as one of the food factors that affect the role of digestion of foods (Wolever, 1990). In a systematic study, it was reported that the addition of phytic acid reduced the contents of RS to a minor extent (Escarpa, Gonzalez, Morales & Saura-Calixto, 1997). Since an enzymatic method of analysis was used, factors known to affect starch digestibility may affect the results obtained.

Results from the present study have shown that the RS content of flour based products could be changed by altering the composition and using different kinds of raw materials. A considerable increase observed in RS content of the wheat bran bread, suggests that a switch from wheat bread to wheat bran bread may increase the total RS intake of Turkish people. Resistant starch has been accepted as a dynamic idea and food ingredient that will enrich our knowledge of carbohydrate chemistry, food science, nutrition and health. In addition to that, resistant starch offers a major advantage over dietary fibre because it can be manipulated technologically to change the dietary fibre content of foods without greatly altering their organoleptical properties.

Acknowledgements

The authors gratefully acknowledge Dr. Hans N. Englyst and Dr. Geoffrey Hudson for providing an Englyst Starch Kit and their invaluable help and contribution to this project.

References

- Alexander, J. A. (1995). Resistant starch — new ingredient for the food industry. *Cereal Foods World*, 40(6), 455–458.
- Annisson, G., & Topping, D. L. (1994). Nutritional role of resistant starch: chemical structure vs physiological function. *Annual Review of Nutrition*, 14, 297–320.
- Asp, N. G. (1996). Dietary carbohydrates: classification by chemistry and physiology. *Food Chemistry*, 57(1), 9–14.
- Berggren, M. Y., Björck, I. M. E., Margareta, E., Nyman, G. L., & Eggum, B. O. (1995). Short chain fatty acid content and pH in caecum of rats fed various sources of starch. *Journal of the Science of Food and Agriculture*, 68, 241–248.

- Björck, I. M. E., & Siljeström, M. A. (1992). *In vivo* and *in vitro* digestibility of starch in autoclaved pea and potato products. *Journal of the Science of Food and Agriculture*, 58, 541–553.
- Cummings, J. H., Beatty, E. R., Kingman, S. M., Bingham, S. A., & Englyst, H. N. (1996). Digestion and physiological properties of resistant starch in human large bowel. *British Journal of Nutrition*, 76, 733–747.
- Cummings, J. H., & Englyst, H. N. (1995). Gastrointestinal effects of food carbohydrate. *American Journal of Clinical Nutrition*, 61(Suppl.), 938S–945S.
- Edwards, C. A., Gibson, G., Champ, M., Jensen, B. B., Mathers, J. C., Nagengast, F., Rumney, C., & Quehl, A. (1996). *In vitro* method for quantification of the fermentation of starch by human fecal bacteria. *Journal of the Science of Food and Agriculture*, 71, 209–217.
- Englyst, H. N. & Cummings, J. H. (1993). Nutritional classification of starch and its validation studies in man. In U. Schlemmer, *Congress of Bioavailability'93, Proceedings Part 1* (pp. 142–145), Karlsruhe.
- Englyst, H. N., & Hudson, G. J. (1996). The classification and measurement of dietary carbohydrates. *Food Chemistry*, 57, 15–21.
- Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*, 46(Suppl. 2), 533S–550S.
- Englyst, H. N., Kingman, S. M., Hudson, G. J., & Cummings, J. H. (1996). Measurement of starch *in vitro* and *in vivo*. *British Journal of Nutrition*, 76, 749–755.
- Englyst, H. N., Veenstra, J., & Hudson, G. J. (1996). Measurement of rapidly available glucose (RAG) in plant foods: a potential *in vitro* predictor of the glycemic response. *British Journal of Nutrition*, 75, 327–337.
- Escarpa, A., Gonzalez, M. C., Morales, M. D., & Saura-Calixto, F. (1997). An approach to the influence of nutrients and other food constituents on resistant starch formation. *Food Chemistry*, 60(4), 527–532.
- Goni, I., Garcia-Diz, L., Manas, E., & Saura-Calixto, F. (1996). Analysis of resistant starch: a method for foods and food products. *Food Chemistry*, 56(4), 445–449.
- Gordon, T. G., Topp, K., Shi, Y. C., Zallie, J., & Jeffcoat, R. (1997). Resistant starch: physical and physiological properties. In M. Yalpani, *New technologies for healthy foods and nutraceuticals*, (pp. 157–178). USA: ATL Press Inc.
- Heaton, M. D., Marcus, S. N., Emmett, P. M., & Bolton, C. H. (1988). Particle size of wheat, maize and oat test meals: effects of plasma glucose and insulin responses on the rate of starch digestion *in vitro*. *American Journal of Clinical Nutrition*, 47, 675–682.
- Holm, J., & Björck, L. (1992). Bioavailability of starch in various wheat-based bread products: evaluation of metabolic responses in healthy subjects and rate and extent of *in vitro* starch digestion. *American Journal of Clinical Nutrition*, 55, 420–429.
- Kingman, S. M., & Englyst, H. N. (1994). The influence of food preparation methods on the *in vitro* digestibility of starch in potatoes. *Food Chemistry*, 49, 181–186.
- Kritchevsky, D. (1988). Dietary fiber. *Annual Review of Nutrition*, 8, 301–328.
- Liljeberg, H., Akerberg, A., & Björck, I. (1996). Resistant starch formation in bread as influenced by choice of ingredients or baking conditions. *Food Chemistry*, 56(4), 389–394.
- Martin, L. J. M., Duman, H. J. W., & Champ, M. M. J. (1998). Production of short chain fatty acids from resistant starch in a pig model. *Journal of the Science of Food and Agriculture*, 77(7), 1–80.
- Sievert, D., & Pomeranz, Y. (1989). Enzyme resistant starch I. Characterization and evaluation by enzymatic, thermoanalytical and microscopic methods. *Cereal Chemistry*, 66(4), 342–347.
- Skrabanja, V., & Kreft, I. (1998). Resistant starch following autoclaving of buckwheat (*Fagopyrum esculentum* Moench) groats. An *in vitro* study. *Journal of Agriculture and Food Chemistry*, 46, 2020–2023.
- Szczodrak, J., & Pomeranz, J. (1992). Starch–lipid interaction and formation of resistant starch in high amylose barley. *Cereal Chemistry*, 69(6), 626–632.
- Türk Standartlar Enstitüsü (1987). *Ekmek Standardı*, 20s.
- Ünal, S. S. (1991). Hububat Teknolojisi, 3.baskı (pp. 114–115). Ege Üniversitesi Mühendislik Fakültesi Coğaltma Yayını, yayın no: 29, Bornova, Izmir.
- Unlu, E., & Faller, J. F. (1998). Formation of resistant starch by a twin-screw extruder. *Cereal Chemistry*, 75(3), 346–350.
- Wolever, T. M. S. (1990). Relationship between dietary fibre content and composition in foods and the glycemic index. *American Journal of Clinical Nutrition*, 51, 72–75.
- Würsch, P., Vedovo, S. D., & Koellreutter, B. (1986). Cell structure and starch nature as key determinants of the digestion rate of starch in legume. *American Journal of Clinical Nutrition*, 43, 25–29.