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Effects of bran size and carob seed flour of optimized bread formulas on glycemic responses in humans: A randomized clinical trial

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ABSTRACT

We investigated the glycemic-index (GI), glycemic-load (GL) and glycemic response to four breads produced by optimized formulas in terms of texture and structure: white bread (WB), bread enriched with coarse wheat bran (CB), with fine wheat bran (FB) and FB with 10% carob-seed flour (CSFB). Ten healthy individuals (24 ± 1 years; BMI 22 ± 3 kg/m^2) received isoglucidic test meals (50 g available carbohydrate) and 50 g glucose reference, in random order. GI/GL was calculated and capillary blood glucose and salivary insulin samples were collected at 0–120 min after meal consumption. CB and CSFB provided medium-GI, low-GL. WB and FB provided high-GI, medium-GL. Peak glucose value was lower for CSFB ($p = 0.03$). Dough water content was inversely associated with GI ($p = 0.03$). No differences were observed between breads for fasting glucose, fasting and post-test-meal insulin concentrations. Larger bran particle size and flour substitution by carob-seed flour attenuated the glycemic response resulting in lower GI or GL breads.

1. Introduction

Worldwide, cereals and their products, especially bread, are the principal components of the human diet. White bread from wheat flour, a high glycemic index (GI) food, is the most widespread and consumed cereal product. White bread's starch is digested and absorbed rapidly from the human digestive system, which may lead to glucose spikes and troughs (Fardet, Leenhardt, Lioger, Scalbert, & Remesy, 2006). Increased glucose fluctuations have been shown to induce oxidative stress and beta-cell damage (Ceriello et al., 2008). Moreover, increased glucose variability from peaks to nadirs has been recognized as a major metabolic defect leading to cardiovascular diseases (Monnier, Colette, & Owens, 2009).

The glycemic index (GI) is a tool that classifies the carbohydrate containing foods according to time integrated effects on postprandial glycemia (FAO/WHO, 1997; ISO). The GI depicts both the standardized and relative postprandial glucose response based on an equal amount of available carbohydrate and relative to a referent food (Augustin et al., 2015). Foods containing carbohydrate that is digested, absorbed and metabolized quickly are considered high GI foods (GI > 70 on the glucose scale) whereas those that are digested, absorbed and metabolized slowly are considered low GI foods (GI < 55 on the glucose scale) (Augustin et al., 2015). The glycemic load (GL) is the product of GI and the total available carbohydrate content in a given amount of food (Augustin et al., 2015). It has been shown that the GL is also a good predictor of the level of postprandial glycemia associated with a particular food (Bao, Atkinson, Petocz, Willett, & Brand-Miller, 2011). Consumption of high GI foods is associated with increased chronic disease risk (Augustin et al., 2015; Barclay et al., 2008; Greenwood et al., 2013); whereas low to moderate GI foods are considered favorable to health (FAO/WHO, 1998). A moderate improvement in glycemic control may be accomplished when low GI foods replace higher GI foods (Evert et al., 2013). Likewise, in cohort studies, the GL, but not the carbohydrate content, has been frequently linked to reduced risk of type 2 diabetes (Livesey, Taylor, Livesey, & Liu, 2013) and cardiovascular diseases (Barclay et al., 2008). It has also been shown that lowering the GL of consumed carbohydrates leads to a significant haemoglobin A1C reduction of −0.2% to −0.5% (Thomas & Elliott, 2009; Wheeler et al., 2012).

Many factors, such as inclusion of soluble dietary fiber (i.e. beta-glucans), resistant starch and amylose, presence of intact or cracked kernels, sourdough fermentation, bread making technology, inclusion of non-cereal ingredients (i.e. fruit fiber, legume-based flours) and flour water content may influence the glycemic response (Augustin et al., 2015; de la Hera, Rosell, & Gomez, 2014; Fardet et al., 2006; Jenkins et al., 2002; Pi-Sunyer, 2002; Ray & Singhania, 2014; Scazzina, Siebenhandl-Ehn, & Pellegrini, 2013; Stamataki, Yanni, & Karathanos, 2017).

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Our group (Papakonstantinou et al., 2017) and others (Milek Dos Santos, Tomzack Tulio, Fuganti Campos, Ramos Dorneles, & Carneiro Hecke Kruger, 2014) have shown that carob (*Ceratonia siliqua*) used in snacks or bars is a low GI and GL ingredient leading to increased satiety, lower energy intake at a following meal and lower glycemic response (Papakonstantinou et al., 2017); possibly due to carob's high soluble fiber content.

Bread formulas in our study were optimized according to the water content required in the dough and its mixing time. The purpose of that approach was to produce breads that could have a commercial impact. Despite of the bran addition in wheat bread we aimed to keep the desired crumb texture.

Breads grain particle size and carob seed flour may affect the glycemic response, but this has not been adequately studied in humans. Moreover, carob seed has a specific importance for Mediterranean agro-food production, as it is rich in protein and fibers.

The aims of this study were: a) to produce 4 types of breads by optimized formulas using response surface methodology (RSM) and then b) investigate the short-term effects of bran particle size and carob seed flour on GI, GL and postprandial glycemic response.

2. Materials and methods

2.1. Experiment 1: Bread formulation

2.1.1. Bread materials

Coarse wheat bran and wheat flour *Triticum aestivum L.*were used (Loulis Mills S.A., Sourpi, Volos, Greece). The wheat flour used was a flour type 45 according to US and French flour categorization with extraction rate of 67–70% and ash content below 0.5%. Fine wheat bran was obtained by grinding some of the coarse bran, with the use of a jet mill (Model 0101S Jet-O-Mizer Milling, Fluid Energy Processing and Equipment Company, Telford, PA, U.S.A.). The grinding process was performed at predefined conditions selected after performing preliminary experiments.

Carob seeds were grounded and then three fractions were separated using sieves (A = 315–500 μm, B = 250–315 μm and C = 125–250 μm). Fraction B was used for bread making in this study. Its protein content is 23.0 ± 0.7 wet basis (w.b.) and its fiber content is 51.8 ± 2.7 (w.b.) (Tsatsaragkou, Kara, Ritzoulis, Mandala, & Rosell, 2017).

Flour particle size, carob, wheat flour and bran, was measured using a laser granulometry (Malvern Mastersizer 2000, Malvern Instruments, Worcestershire, UK) equipped with a Scirocco dry powder unit (Malvern Instruments, Worcestershire, UK). The instrument provides volume-weighted size distributions.

The ingredients for white bread (WB) (300 g flour, 9 g yeast, 6 g salt and water) were weighted and mixed in a Hobart mixer (N50, Hobart Co., USA). For the coarse bran bread (CB), 240 g white flour and 60 g coarse bran were used (20% substitution of the initial amount of flour), whereas 58 g fine bran was used for the fine bran bread (FB). Bran milling resulted in moisture loss, therefore a small compensation was made for fine bran, to achieve the same amount of dry matter. On a dry basis 53 g of bran was used in all formulations. For the carob seed flour – fine bran bread (CSFB) 210 g white flour, 58 g fine bran and 30 g carob seed flour were used. In this sample, a substitution level of the initial flour was at 30%, keeping constant bran level at 20% and adding 10% carob flour. By that formulation we wanted to investigate a combined effect of bran and carob.

Small loaves (70 g) were made and fermented for 70 min at a Memmert oven (30 °C). Then, they were baked in an electric oven for 15 min at 180 °C. After baking, they were cooled for 1 h at room temperature (23 °C), and then the measurements were made.

2.1.2. Bread experimental design

Specific volume (SV, ml/g) was measured by a volumetric displacement method using solid-glass beads with 2 mm diameter. Hardness was determined with an Instron texture analyzer (Universal Testing Machine, Model 1100, USA). Cubes (2 × 2 × 2 cm) from the centre of the loaf were compressed at depth 40% of the original height, at a crosshead speed of 101 mm/min. The max load (N) was measured. For the evaluation of crumb's morphological characteristics, 1 cm thickness slices were obtained from the centre of the loaf and images were taken with a flatbed scanner (HP Scanjet 4370, Hewllett-Packard, USA). Porosity (%) and max diameter (cm) were calculated using Image analysis software (ImageProPlus 7, Media Cybernetics, USA). Porosity was measured as the surface of pores/ total surface of the crumb, which was defined to 4 cm^2 per slice.

Available carbohydrates and dietary fibre were determined according to the method AOAC 991.43, with the Megazyme assay kit (Bray, Ireland). In short, this method involves digesting the food with appropriate enzymes and photometric determination of free sugars. Moisture determination was based on the method AACC 44-15A (1999).

Coarse bran bread's and fine bran bread's quality parameters were optimized using response surface methodology. A central composite design was applied with two factors at three levels. Variable is called a factor and each factor had three different values. Variables were selected according to the composition of the breads. Water was selected as a variable, knowing that the increased amount of fibers results in high water levels in the recipe. Moreover, mixing time was selected as a variable taking into account the particle size of the bran fractions and the differences in the composition of the dough. Variable range for each one was selected after a series of screen tests. The central point was repeated, leading to a total of 10 experiments for each of the two breads (3^2+1). Each response variable of the model was related to the independent variables of the experiment according to the following polynomial function:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2 \quad (1)$$

The response variables (y) were: the specific volume of bread (SV), crumb firmness (Max Load, hardness), the maximum diameter of crumb pores (Dmax) and the surface porosity of the crumb (Porosity). The independent variables were: water – (X_1) and mixing time: (X_2)

2.1.3. Bread products

Four different breads were made using the best formulations according to the experimental design results: white bread (WB) containing 60% water; bread enriched with coarse wheat bran (CB; 65% water, 11 min mixing time), bread enriched with fine wheat bran (obtained by milling of the coarse bran; FB; 65% water, 11 min mixing time) and a FB in which 10% of the white flour was substituted by carob seed flour (CSFB). In this bread 92% of water was added according to preliminary experiments and results and mixing time was kept at 11 min (Tsatsaragkou K. et al., 2017).

2.2. Experiment 2: Clinical trial in healthy humans

2.2.1. Subjects

Healthy, non-smoking, non-diabetic, men and women participated in this randomized, blind, crossover clinical trial. Subjects were chosen via notices at the Agricultural University of Athens. The inclusion criteria were a body mass index (BMI) between 18 and 32 kg/m^2 and age between 18 and 50 years old. Exclusion criteria included chronic diseases (e.g. coronary heart disease, diabetes mellitus, liver or renal disease), gastrointestinal disorders, pregnancy, lactation, attending competitive sports and high alcohol consumption. Ten participants fulfilling all inclusion criteria completed all endpoint assessments. All subjects gave their written consent. The protocol was approved by the Bioethics Committee of the Agricultural University of Athens and was carried out in accordance with the Declaration of Helsinki (1997). ClinicalTrials.gov Identifier: NCT03314142.