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# *In vivo* study on the slow release of glucose in vacuum fried matrices

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ABSTRACT

*In vitro* studies have shown that vacuum frying may be an effective process to reduce starch digestibility as it may limit gelatinization; this is significant as overconsumption of starchy foods contributes to obesity and type 2 diabetes. Although *in vitro* studies are an instrumental tool, *in vivo* studies allow observation of the overall effect on a living organism. The aim of this research was to assess how *in vivo* starch digestibility can be reduced when frying under vacuum (9.9 kPa), after feeding Sprague-Dawley rats, while also understanding its relationship to *in vitro* starch digestibility. Results showed that vacuum-fried dough has a lower degree of gelatinization (~53.8%) and a maximal blood glucose level at 60 min (slower glycemic response) than atmospheric counterparts (~98.3% degree of gelatinization and maximal blood glucose level at 30 min). Similarly, *in vitro* procedures exhibited less rapidly available glucose and higher unavailable glucose fractions in vacuum-fried dough.

## 1. Introduction

High consumption of starchy foods is associated with type 2 diabetes, which has developed into a worldwide epidemic (Bowman et al., 2004). Furthermore, type 2 diabetes is also closely linked with obesity, as obese individuals often develop exacerbated insulin resistance (Zimmet, Alberti, & Shaw, 2001). Consequently, diet is recognized as a modifiable risk factor for this type of diabetes (Tachibe, Kato, Sugano, Kishida, & Ebihara, 2009). As changing the dietary habits of a population is complex, efforts should be directed to increasing the content of resistant starch in foods (Sajilata, Singhal, & Kulkarni, 2006). In fact, even though the nutrient composition of a food can be precisely estimated, the availability of nutrients for absorption in the gut is often quite uncertain or varies for the same food depending on processing conditions, the presence of other components, and other variables (Parada & Aguilera, 2007). When starch granules are gelatinized, the disruption of their structure increases their susceptibility to enzymatic degradation and related digestibility (Holm, Lundquist, Björck, Eliasson, & Asp, 1988). However, if they remain ungelatinized, a form of resistant starch, they have been shown to resist hydrolysis (Birt et al., 2013; Sajilata et al., 2006).

To measure these differences, Englyst, Englyst, Hudson, Cole, and Cummings (1999) developed an *in vitro* enzymatic procedure to classify starch digestibility. Accordingly, rapidly available glucose (RAG) is defined as the fraction that is obtained after 20 min of hydrolysis, while slowly available glucose (SAG) is the fraction obtained between 20 and 120 min of hydrolysis. Lastly, unavailable glucose (UG) is defined as the fraction that cannot be released after 120 min. Using this methodology, Parada and Aguilera (2009), found that an increased proportion of gelatinized granules led to higher *in vitro* digestibility in starch in water suspensions that were heated to obtain different degrees of gelatinization.

Several starchy foods may have a portion of remaining starch that is not fully gelatinized during processing, due to water accessibility limitations or insufficient temperature. Such foods may include cereal flakes and baked products, due to the high heating rates involved in the process (Rashmi & Urooj, 2003; Venn & Mann, 2004). In recent research, Contardo, Parada, Leiva, and Bouchon (2016) found that vacuum frying, a deep-fat frying process that is carried out under pressures well below atmospheric pressure, may impair *in vitro* starch digestibility. The processing conditions can markedly decrease the boiling point of water (e.g. 38 °C), allowing to reduce the processing (oil) temperature (e.g. 108 °C). The temperature of the crust region, which does not contain liquid water anymore, may rise above the boiling point of water (Bouchon & Pyle, 2005). However, the temperature of the core region, which contains liquid water, is restricted to the boiling point of water, which is below to the one needed for starch gelatinization (~60 °C). Therefore, the availability of liquid water at a sufficiently high temperature to induce starch gelatinization becomes limited, reducing starch digestibility.

Although *in vitro* studies are an instrumental tool to assess starch digestibility using simulated gastro-intestinal conditions, they may lead to results that do not correspond to real circumstances. For this reason, *in vivo* studies are often employed over *in vitro* tests since they allow the

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overall effect of the experiment to be observed on a living organism. A key goal is to get a good understanding of the relationship between *in vitro* and *in vivo* tests, as has been targeted in other studies in different products (Singh, Dartois, & Kaur, 2010). In this context, *in vitro* studies have shown that partially gelatinized starch can be expected to cause a slow and prolonged release of glucose and a moderate glycemic response compared to gelatinized native starch (Juansang, Puttanlek, Rungsardthong, Puncha-Arnon, & Uttapap, 2012). With respect to the effect of starch gelatinization, *in vivo* studies have shown that glucose response and insulin response are positively correlated with the degree of starch gelatinization (Chung, Lim, & Lim, 2006). In addition, both responses have shown to have a positive correlation with the rate of hydrolysis using α-amylase (Holm et al., 1988).

Consequently, the aim of this research is to assess how *in vivo* starch digestibility can be reduced when deep-fat frying under vacuum conditions, while also understanding its relationship to *in vitro* starch digestibility.

## 2. Materials and methods

### 2.1. Materials

Dough was prepared using vital wheat gluten (Asitec S.A., Santiago, Chile), native wheat starch (Comercial Venser S.A., Santiago, Chile), and distilled water, to have an appropriate control of ingredients proportion (Gazmuri & Bouchon, 2009). Samples were fried in high oleic sunflower oil (Camilo Ferrón Chile S.A., Santiago, Chile).

Pepsin-P7000, amyloglucosidase-A7095, pancreatin-7545 (Sigma-Aldrich, St Louis, MO, USA), and invertase-390203D (VWR International Ltd., Poole, UK) were used to perform *in vitro* digestibility essays.

Glucose (Sigma-Aldrich, St Louis, MO, USA) and indigo carmime (Sigma-Aldrich, St Louis, MO, USA) were used in the *in vivo* digestibility studies.

### 2.2. Sample preparation

Dough was prepared according to Contardo et al. (2016), using native wheat starch (88% dry basis) and wheat gluten (12% dry basis), ensuring 40% final moisture (wet basis) in all samples. The dough was sheeted using a LSB516 dough sheeter (Doyon, Saint-Côme-Linière, Quebec, Canada), obtaining a final thickness of 2 mm. The sheeted dough was cut into squares ($3.8 \times 3.8\ cm^2$), which were stored in plastic film to prevent dehydration.

### 2.3. Atmospheric and vacuum deep-fat frying experiments

All frying experiments were performed using the same equipment, an electrically heated 10-L stainless steel fryer that can be hermetically covered with a stainless steel lid, following the procedure described by Contardo et al. (2016) and with few modifications. The fryer was filled with 3.5-L of high-oleic sunflower oil preheated for 1 h before frying. A thermal driving force of 70 °C ($\Delta T = T_{oil} - T_{boiling\ point\ of\ water\ at\ working\ pressure}$) was used to compare atmospheric and vacuum frying (Mariscal & Bouchon, 2008). The boiling point of water was 45 or 100 °C, under vacuum (9.9 kPa) or atmospheric conditions, respectively. This yielded frying temperatures of 115 and 170 °C, respectively.

After the oil reached the frying temperature ( ± 2 °C), only 8 slices (~30 g) of dough were fried at a time. In vacuum frying experiments, the samples were loaded and the vessel was depressurized. The fryer basket was then immersed into the oil for the required period of time. Samples were fried up to bubble-end point (≈2.5% moisture content), a period of time defined as $t_{ep}$, or up to half this time ($t_{hp} = t_{ep}/2$). $T_{ep}$ and $t_{hp}$ were 180 and 90 s in vacuum frying experiments, and 240 and 120 s in atmospheric frying experiments, respectively.

### 2.4. Analytical methods

#### 2.4.1. Moisture content

Moisture content was determined gravimetrically after drying in a forced oven at 105 °C up to constant weight (official method 945.15; AOAC, 1995).

#### 2.4.2. Oil content

The oil content of the dry samples was determined gravimetrically by Soxhlet extraction using petroleum ether (official method 920.39; AOAC, 1995).

#### 2.4.3. Starch gelatinization degree

The starch gelatinization degree was determined according to Contardo et al. (2016), using a Mettler Toledo 821 DSC (Mettler-Toledo Inc., Schwerzenbach, Switzerland). Before DSC analysis, the raw samples (unprocessed dough with 40% moisture) were dehydrated at 40 °C for 24 h, until they reached ~3% moisture (dry basis). All samples were then ground up and sieved (60-mesh sieve). Approximately 4 mg of dry sample was placed in a DSC aluminium pan and distilled water was added to yield a water to starch ratio of 4:1. The pan was hermetically sealed and kept in room temperature for ~12 h. The samples were heated at 10 °C/min from 35 to 90 °C. The gelatinization enthalpies of the raw ($\Delta H_{Raw}$) and the fried samples ($\Delta H_{Fried}$) were computed, and the degree of starch gelatinization (DG) was determined according to Eq. (1):

$$DG(\%) = \left(\frac{\Delta H_{Raw} - \Delta H_{Fried}}{\Delta H_{Raw}}\right) \times 100 \quad (1)$$

#### 2.4.4. Water absorption index

The water absorption index (WAI) was determined as described by Anderson, Conway, and Peplinski (1970) with some modifications. Prior to analysis, the samples were ground and sieved using a 12-mesh sieve. Subsequently, 2.5 g of sample were hydrated using agitation during 30 min at 37 °C, under water-excess conditions, keeping a sample at a water ratio of 1:12. The solution was centrifuged (3000*g*) for 10 min. The supernatant was discarded and the remaining precipitate was weighted to determine the amount of absorbed water per gram of dry sample. The WAI (g water/g dry solids) was determined according to Eq. (2):

$$WAI = \frac{g\ absorbed\ water}{g\ dry\ solids} \quad (2)$$

This procedure was repeated, replacing the distilled water with two buffer solutions (pH = 3 and pH = 11), in order to measure the extent of water absorption at different pHs (3, 7, and 11).

#### 2.4.5. Texture analysis

Texture of samples was analysed as described by Dueik, Robert, and Bouchon (2010), using a three-point bending test, mounted in a TA.XT Plus Texture Analyzer (Stable Micro Systems Ltd, UK), considering a support span of 16 mm. A 2.5 mm-thick steel blade with a flat edge was used to fracture the sample at a moving speed of 10 mm/s. The maximum breaking force ($F_{max}$) at the fracture point (highest value in the plot) was obtained using Texture Expert software version 1.16. Texture analyses were carried out in 16 replicates for each frying condition.

### 2.5. In vitro starch digestibility

*In vitro* starch digestibility was determined according to Contardo et al. (2016) with few modifications. During the first stage, approximately 1.5 g of sample was mixed in polypropylene tubes with 5 mL of a 50% saturated benzoic solution and 10 mL of pepsin guar-gum solution (5 g pepsin/L and 5 g guar gum/L in 0.05 M HCl). Guar gum was added