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# The cytotoxic effect of artificially digested buckwheat products on HT-29 colon cancer cells

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ABSTRACT

Recently Europeans are showing a growing interest in healthy foods. In light of this fact, the aim of our study was to evaluate the effect of buckwheat products (groats, hull, and bran) after *in vitro* digestion in an artificial gastrointestinal tract on the inhibition of HT-29 cancer cell proliferation. We investigated the content of individual flavonoids, phenolic acids, amino acids, and thiamine in these buckwheat products. The MTT (thiazolyl blue tetrazolium bromide) assay was used to evaluate the viability and metabolic activity of the colon cancer cells. We found significant ($p < 0.05$) negative correlations between HT-29 cell growth and the levels of catechin, quercetin, serine, proline, glycine, histidine and arginine in buckwheat samples after digestion. Our results highlight that buckwheat by-products are a rich source of bioactive substances such as: quercetin, catechin, serine, proline, glycine, histidine, and arginine. Therefore, buckwheat can be used as an additive in pro-health foods.

## 1. Introduction

Colon cancer is a major cause of cancer morbidity and mortality and in 2012 alone, it accounted for 694000 deaths worldwide. The risk of developing this type of disease is higher in older patients, especially those over the age of 60. The incidence of cancer is much lower among vegetarians, or those individuals who do not consume meat or dairy products (Coulston et al., 2017). In fact bioactive compounds present in fruits and vegetables are known to have beneficial effects on human health, especially in protecting against chronic diseases, such as cardiovascular diseases and cancer (Coulston et al., 2017). These benefits may be associated with bioactive compounds like dietary fibre, aminoacids, phytosterols and other accompanying substances such as phenolic components (Coulston et al., 2017).

Cereals and pseudocereals are an important source of macronutrients and bioactive substances that have antioxidative activity. Buckwheat (*Fagopyrum esculentum*) grains and groats contain numerous flavonoids and polyphenols, such as: rutin, catechin, quercetin, *p*-coumaric acid, *p*-hydroxybenzoic acid and gallic acid. Furthermore, buckwheat grains contain proteins of high biological value, a well-balanced amino acid composition, a relatively high dietary fibre content and the vitamins B1, B2 and B6. Phenolic antioxidants can inhibit free radical formation and protect against tissue damage that is associated with various human diseases. Antioxidative activity is most often attributed to phenolic acids, flavonoids and phytosterols (Senthilkumar et al., 2013).

Ishii et al. has shown that buckwheat hull extract has a strong antioxidative effect both *in vitro* and *in vivo* (Ishii et al., 2008).

Although the inhibitory effect of polyphenols on the growth of different cancer cells *in vitro* is well described, the effect of individual antioxidative substances on cultured cells after *in vitro* digestion has not been explored (Li et al., 2016; Sathiyamoorthy and Sudhakar, 2018). Antioxidants are responsible for, among others functions, free radicals scavenging, singlet oxygen scavenging, and the removal of electrons in cellular redox reactions.

The transmembrane potential of living cells is capable of transporting MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) across the membrane to the inside where it forms water-insoluble formazan crystals (Liu and Nair, 2010). Therefore the conversion of mitochondrial tetrazole to formazan is dependent on variable

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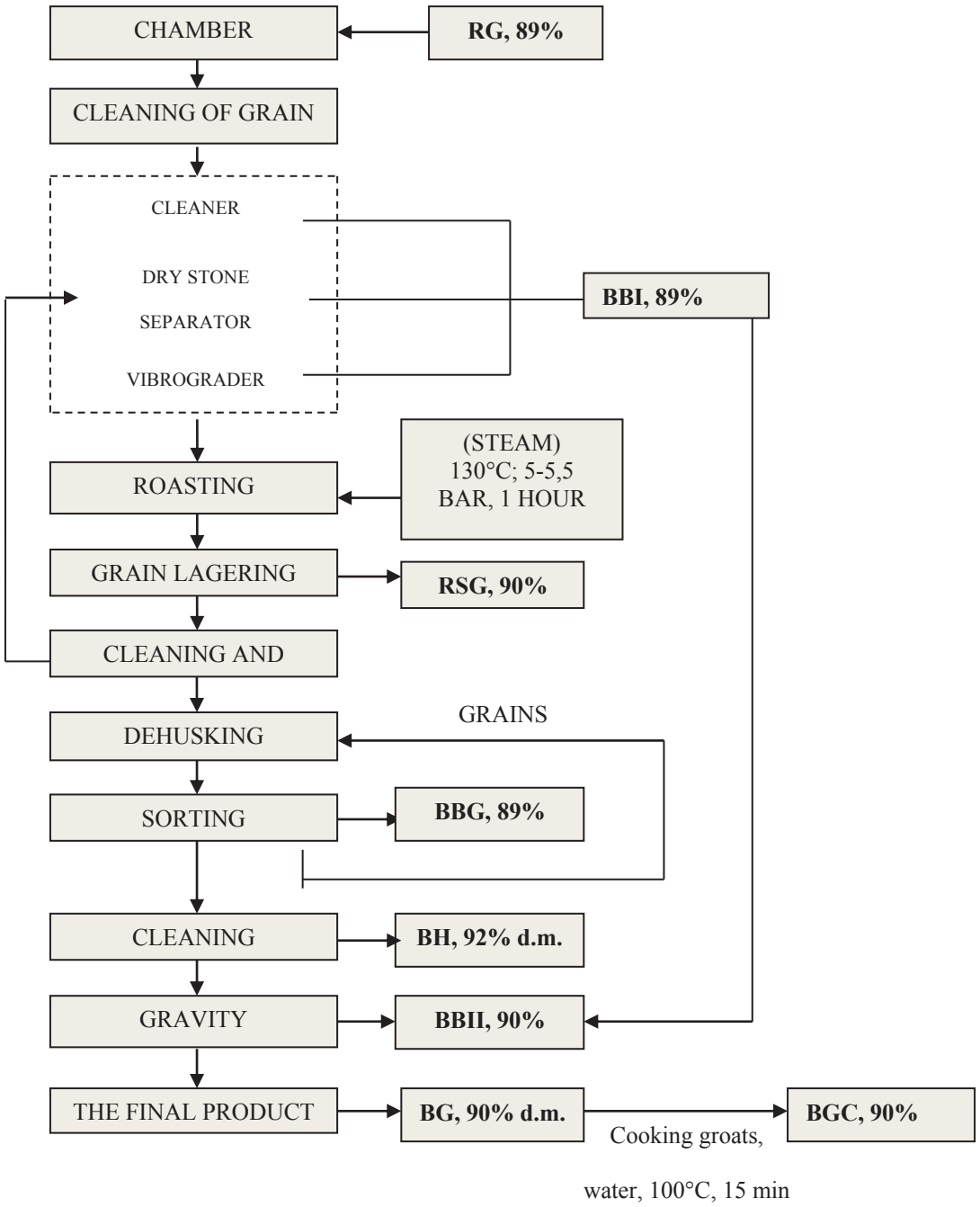


**Fig. 1.** Technological process of buckwheat groats production "Podlaskie Zakłady Zbożowe"- milling company Białystok, Poland.
RG-raw grains; RSG-roasted grains; BG-buckwheat groats; BGC-cooked groats; BBG-broken groats; BBI-buckwheat bran I; BBII-buckwheat bran II; BH-buckwheat hull, d.m.-dry matter.

cells. In this respect, using the MTT assay to measure the redox reaction in cancer cell lines treated with digested buckwheat samples can provide reliable observations.

Here, we aimed to identify which type of buckwheat product has the greatest cytotoxic effect on colon cancer cells. For this purpose, we cultured colon cancer cells and treated them with different products that were previously digested in an artificial gastrointestinal tract.

## 2. Materials and methods

### 2.1. Materials

Buckwheat products (*F. esculentum* Moench) - raw grains (RG), roasted grains (RSG), buckwheat (whole) groats (BG), cooked groats (BGC), broken groats (BBG), first stage of processing buckwheat bran (BBI), second stage of processing buckwheat bran (BBII) and buckwheat hull (BH) - were obtained from the commercial food company "Podlaskie Zakłady Zbożowe", Białystok, Poland (Fig. 1).

### 2.2. In vitro digestion

A simulation of the human digestive system was carried out according to Dziedzic et al. on 30 g of buckwheat samples (Dziedzic et al., 2015). The environment of the stomach and small and large intestine was reproduced as closely as possible. The digestive model did not include absorption of bioactive components, because of the difficulty in