Natural sources of health-promoting starch

DANIELA MIKULÍKOVÁ - JÁN KRAIC

Summary
Resistant starch (RS) is considered a dietary fibre and prebiotic provided by functional foods. It has many proven health-promoting benefits, particularly in the prevention of cancer and cardiovascular diseases. Commercially available functional foods usually contain chemically-modified resistant starch types RS_4 or RS_2 derived from genetically-modified plant lines with high amylose content. The aim of this study was to screen for natural sources of RS_3 (arising during cooking, baking and freezing) in cereals, pseudocereals and legumes. Highly valuable natural sources of health-promoting RS_3 resistant starch were: triticale (cultivars Kendo, Pinokio, Presto, Tricolor), rye (Dankowskie Nove, Esprit), wheat (Boka), buckwheat (Pyra), chickpea (Alfa), kidney bean (Fabia, Ultima), pea (Elkan, Gloriosa, Zázrak z Kelvedonu), faba bean (Omar) and lentil (Renka).

Keywords
resistant starch; functional foods; cereals; pseudocereals; legumes

The idea that human health is affected by food is very old. Long ago Hippocrates (460–377 A.A.C.) wrote in his medical documents: "Each food component acts on the human body and changes it. Future human life depends upon these changes and there will be either health, disease or healing". Another well known saying is: "Let food be the medicine".

Concepts regarding food function for human life, were modified in stages. In the past, the functions of hunger satiation and life sustenance were emphasized. Later, it was recognized that disease developed in the absence of nutrients. Currently, attention is being paid to foods that improve wellness. In effort to prevent the civilization diseases, increased attention has been given to functional foods. They fall somewhere between everyday foods and medicines. Over the past 20 years, functional foods have been preferred in Japan (FOSHU - Foods for Specific Health Uses), as well as presently, in countries of the Europe Union (PARNUTS - Foods for Particular Nutritional Uses). These foods also possess, in addition to their nutritional value, a beneficial effect on human health, mental alertness and physical performance. Functional foods are foods to which a component has been added for a resultant positive health effect or eliminated due to a deleterious effect on health. [1]. Consumption of functional foods is recommended for their preventative benefits. Functional foods must remain foods and at the same time the beneficial effects from their consumption must be evident after consumption of portions which are commonly consumed. Among the added components are: probiotics (bifidobacteries), prebiotics (resistant starch, β-glucan, oligosaccharides, pectins), vitamins (B_6, B_{12}, D, K, folic acid), minerals (Ca, Mg, Zn), antioxidants (vitamin E and C, carotenoids, flavonoids), fatty acids (ω-3 fatty acids, γ-linolenic acid, conjugated linoleic acid), proteins, peptides, aminoacids and phytochemicals (phytosterols, isoflavons, lignans). Among the components removed from functional food are: food allergens, pathogenic microorganisms, mycotoxins, pesticide residuals, and heavy metals.

One of components of functional foods is resistant starch (RS). The RS latter is defined as sum of starch and starch degradation products not absorbed in the small intestine of healthy man [2]. There are four types of RS: starch that is physically inaccessible for degradation by amylolytic enzymes of the digestive tract (RS_1); starch with a primary structure and conformation that results in its natural resistance to degradation, such as the green

Daniela Mikulíková, Research Institute of Plant Production, Bratislavská cesta 122, SK-921 68 Piešťany, Slovakia.
Ján Kraic, Research Institute of Plant Production, Bratislavská cesta 122, SK-921 68 Piešťany, Slovakia; University of ss. Cyril and Methodius, Nám. J. Herdu 2, SK-917 01 Trnava, Slovakia.

Correspondence
Daniela Mikulíková, e-mail: mikulikova@vurv.sk

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banana, crude potato or plant cultivars with high amylose content (RS_2); retrograded resistant starch (RS_3); and chemically-modified starch (RS_4).

Due to its beneficial effects on digestive physiology, RS is regarded as a prebiotic among the new generation of dietary fibre [3, 4]. RS creates a barrier with protective and nutritive effects in the colon. Undigested, it passes from the small to the large bowel and undergoes fermentation by enteral microflora (especially *Fussobacterium* spp. and *Clostridium* spp.). During the fermentation process, natural short-chain fatty acids - SCFAs (butyrate, propionate, acetate) and gases (carbon dioxide, methane and hydrogen) are formed. These acids, by themselves, mediate health-promoting effects. RS has a preventative effect against the development of ulcerative colitis [5], as well as against colorectal cancer [6]. A prolonged diet high in resistant starch has protective benefits in healthy individuals against the development of non-insulin dependent diabetes mellitus as well as cardiovascular diseases [7]. RS-rich food has a low glycaemic index and maintains normal levels of glucose, insulin, cholesterol and triacylglycerols in human blood [8-10].

Resistant starch was first commercially manufactured in Australia in 1993 from a maize-hybrid with more than 80% amylose. Thereafter, a technique was patented for its production in the USA. It can be added to bread and other baking products, pasta, nutrition bars, breakfast cereals, yoghurt, puddings, instant soups, sauces, beverages and similar products.

Many well-known corporations (Cerestar, American Maize, OJI Starch, Avebe) produce functional foods enriched with resistant starch. Production methods use either chemically-modified type RS_4 or type RS_2 obtained from genetically-modified corn or barley cultivars. At this time in Slovakia, such foods as well as resistant starch as a nutritional supplement are not available. Currently, no data are published regarding retrograded RS_3-enriched functional foods produced from non-genetically modified plant sources with naturally occurring high amylose starch content. To date, RS_3 content within individual plant crops and their cultivars has yet to be examined.

MATERIAL AND METHODS

Seeds from analyzed crops were obtained from the genetic plant department collection at the Research Institute of Plant Production in Piešťany, Slovakia and the Research Institute of Crop Production in Praha-Ruzyně, Czech Republic. Seeds harvested in the year 2003 were analyzed. The evaluated set contained seed samples from 248 cultivars from 18 plant crops: 26 wheat cultivars (*Triticum aestivum* L.), 31 barley (*Hordeum vulgare* L.), 11 rye (*Secale cereale* L.), 13 triticale (X *Triticosecale Wittm.*), 9 tritordeum (*Tritordeum Aschers & Graebn*), 12 oat (*Avena sativa* L.), 15 buckwheat (*Fagopyrum esculentum* MOECH), 18 millet (*Panicum milliaceum* L.), 10 foxtail millet (*Setaria italica* (L.) P. BEAUV), 6 sorghum (*Sorghum bicolor* (L.) MOENCH), 2 quinoa (*Chenopodium quinoa* Willd.), 25 amaranth (*Amaranthus* L.), 20 pea (*Pisum sativum* L.), 11 lentil (*Lens esculenta* MOENCH), 14 kidney bean (*Phasoleum vulgaris* L.), 5 faba bean (*Vicia faba* L. (partim), 16 chickpea (*Cicer arietinum* L.) and 8 soya bean (*Glycine max*. (L.) MERRILL).

Before chemical analysis an optimization of plant material treatment for creation of retrograded RS_3 [11] was performed to ensure accuracy and reproducibility of determined values and for the preparation of internal control samples for each plant crop. The measurement of resistant, soluble (non-resistant) and total starch was performed in two duplicate determinations using a Resistant Starch Assay Kit and Resistant Starch Control Flours (Megazyme Int., Bray, Ireland) based on the McCleary et al. method [12]. This method is accepted by the AACC (Method 32-40) and the AOAC (Method 2002.02) [13]. A standard error of ± 5% is routinely achieved. The results were calculated for dry weights. Statistical significance within each crop-plant was evaluated by analysis of variance. The relationship between resistant and total starch content in peas was evaluated by means of correlation coefficient.

RESULTS AND DISCUSSION

Cereals

Resistant starch content ($g.kg^{-1}$ dwb) for individual cereals was: triticale 52.8 ± 6.8, rye 49.3 ± 7.3, wheat 39.5 ± 5.7, spring barley 25.1 ± 2.5, winter barley 23.5 ± 4.5, tritordeum 22.6 ± 3.6 and oat 4.1 ± 0.9. The proportion of resistant/total starch was: triticale 7.59%, rye 7.01%, wheat 5.64%, spring barley 3.97%, winter barley 3.89%, tritordeum 3.59% and oat 0.86%. Crops containing more than 4.5% RS are considered to be a suitable source.

The highest level of RS_3 was identified in triticale (wheat × rye). In some triticale cultivars (Pinokio, Presto, Tricolor, Kendo) a higher RS_3 content was detected in comparison with rye (Selgo, Esprit, Dankowskie Nove, Apart). The results are consistent with clinical study outcomes in which the consumption of a standard dose of rye bread had similar effects as resistant starch - improved postprandial glucose levels and insulin response [14, 15]. Total starch levels in triticale and rye reached almost 70% and were similar to that of wheat.

It is evident that wheat is also a relatively superior source of natural RS_3 (Fig. 1). Set of 26 wheat cultivars of various baking quality was specifically selected (E - elite, A - good quality, B - bread quality, C - low bread-baking quality). The highest RS_3 was found in Athlet, Trane, Versailles, Torysa (all C-quality), and Boka (good bread-baking B-quality). The highest average level of RS was in C-quality wheats. Their starch showed higher amylose content. The lowest RS_3 was found in elite (E) wheats - Brea, Ebi, Ilona. In recent studies, the negative effect of higher amylose content on wheat baking quality has been confirmed [16, 17]. Our results indicate that the Boka cultivar is especially advantageous - despite its high RS_3 level and good bread-baking B-quality.

Lower resistant and total starch levels were found in barley in comparison to triticale, rye, and wheat. The highest level of RS_3 was found in the Kompakt, Jubilant, Karát (spring barley), Luxor, and Hanna (winter barley) cultivars. No statistically significant differences were found in resistant and total starch between spring and winter barley or between barley of malting (A) and feed (C) quality. For comparison, we also analyzed barley mutant Glacier (obtained from Washington State University) which is known to have a high amylose content and similar health-promoting benefits as RS [18, 19]. The mutant contained RS_3 51.7 $g.kg^{-1}$ groats. Total starch levels were substantially lower in comparison with other barley cultivars. The resistant/total starch proportion was 9.42%.

In tritordeum (wheat × barley) accessions, resistant and total starch levels were lower than in wheat and similar to those in barley.

The lowest content of both starches and the greatest variation in total starch levels were detected in oat. Total starch content in the naked oat Ábel, Detvan, Izak and Jakub (currently renamed Avenuda) was on average significantly higher in comparison with mean values for all oat cultivars.

The lowest levels of RS_3 were identified in barley and oat. These plant crops are rich in β-glucan [20, 21]. In agreement with our results, EHRENBERGEROVÁ et al. [22] have described a negative correlation between starch amylose and β-glucan levels. FLAMME et al. [23] found highest β-glucan level in barley *waxy* varieties. These varieties with null allele of *waxy* gene are deficient in granule-bound starch synthase, which is responsible for amylose synthesis in starch. The starch of these barleys consists of high amylopectin and very low amylose level, from which resistant starch through retrogradation is created. The occurrence of this phenomenon remains to date, not yet elucidated.

Pseudocereals

Resistant starch RS_3 levels ($g.kg^{-1}$ dwb) were as follows: buckwheat 37.9 ± 3.6, millet 28.7 ± 1.6, foxtail millet 28.4 ± 8.7, sorghum 16.3 ± 3.2, quinoa 12.6 ± 1.29, and amaranth 12.4 ± 12.2. Resistant/total starch proportions were: buckwheat 6.51%, foxtail millet 4.59%, millet 4.37%, sorghum 2.35%, quinoa 2.18%, and amaranth 1.98%.

The highest content of RS_3 was found in buckwheat (Fig. 2), and particularly in La Harpé, Bogatyr, Pyra, and Kora cultivars. The fact that this crop contains high resistant and low total starch levels, demonstrates that it contains more amylose. Simi-

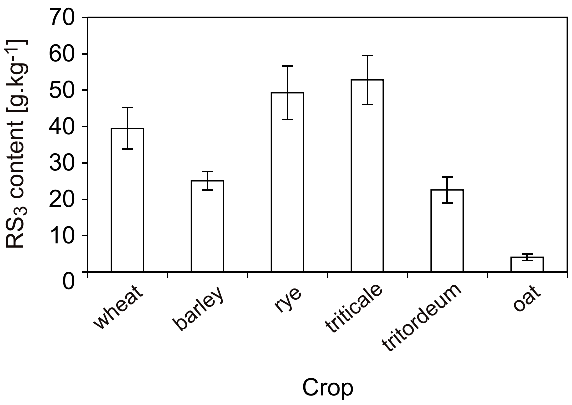

Fig. 1. Content of resistant starch RS_3 in cereals [$g.kg^{-1}$ dwb].

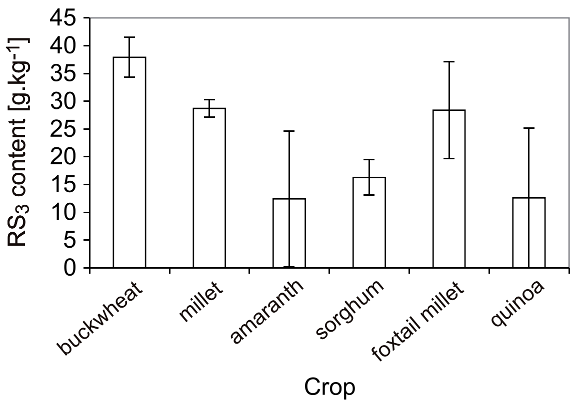

Fig. 2. Content of resistant starch RS_3 in pseudocereals [$g.kg^{-1}$ dwb].

lar results have also been obtained by other authors [24-26].

In comparison with buckwheat, millet was found to have lower resistant and more total starch content. Maximum levels were detected in Voronežskoje 420 and ABY-BS 4091.00.

In foxtail millet, RS_3 and total starch levels were similar to millet. The highest RS was found in the Sibirske klasnate and Sovietzka cultivars. However, in this crop a higher variability in starch amylose was found.

In sorghum cultivars, total starch levels were the highest, however, RS_3 levels were low.

Due to a lack of availability, only two quinoa cultivars were evaluated. The level of health-promoting resistant starch in this crop was low. LINDEBOOM et al. [27] evaluated starch content and composition in 16 quinoa cultivars. The authors found a very low amylose content, fluctuating in the range of 4–20%. It is clear therefore, that quinoa does not posses the conditions for the formation of retrograded RS_3.

The largest variation in RS_3 was demonstrated in 18 evaluated amaranth accessions. Evaluated amaranth types were: *hybridus*, *powellii*, *ssp*, *hypochondriacus*, *cruentus* and *paniculatus*. Only six from 25 analyzed cultivars contain higher RS_3 content: PI 604672, PI 604671, Metelcatyj, A 47, AMAR-2R-R 158, and DF 111. Our results were consistent with the amaranth waxy nature described in the literature [28, 29]. It was discovered that amaranth starch contains mainly amylopectin (above 78%). Amylose content constitutes between 0–22%, but the majority of amaranths have about 10% [30]. The low amylose content in amaranth starch results in its physicochemical properties. In comparison with wheat and maize, amaranth starch is more efficient in binding water and has a higher water solubility. On the Brabender viscograph it shows only a slow tendency to retrogradation [31]. Our results for total starch are similar to those of other authors [32]. Total starch level was higher in waxy amaranths (average 641.1 $g.kg^{-1}$) than in high amylose amaranths (587.9 $g.kg^{-1}$).

In spite of the low occurrence of high amylose amaranths, some cultivars expressed health-promoting benefits in animal models similar to resistant starch - a reduction in glucose, triacylglycerols and LDL as well as total-cholesterol [33-35].

Legumes

There is more resistant and less total starch in legumes (excluding soya beans) in comparison to cereals and pseudocereals. It has been documented that leguminous starches contain more amylose in comparison with cereals and pseudocereals [36].

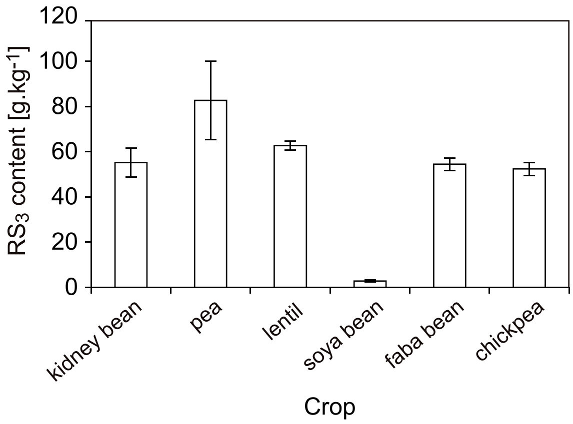

Fig. 3. Content of resistant starch RS_3 in legumes [$g.kg^{-1}$ dwb].

The mean RS_3 content was as follows: kidney bean 55.1 ± 6.4, pea 82.7 ± 1.73, lentil 62.7 ± 2.0, chickpea 52.4 ± 2.9, faba bean 54.4 ± 2.8, and soybean 2.8 ± 0.4 $g.kg^{-1}$ dwb (Fig. 3). The resistant to total starch proportions were: kidney bean 12.66%, pea 21.27%, lentil 11.92%, chickpea 11.04%, faba bean 12.34%, and soybean 4.89%

Pea contains the most RS_3 of all 18 plant-crops evaluated. In this vegetable type, we identified a large variability in resistant and total starch content among cultivars. These were divided into two groups, namely smooth and wrinkled seed peas. A significant difference in resistant and total starch was observed in both groups. The wild pea (smooth seed) had higher total starch (479.2 ± 8.0 $g.kg^{-1}$) and lower RS_3 levels (64.6 ± 5.5 $g.kg^{-1}$). Its resistant/total starch proportion was 13.48 ± 1.09%. Wrinkled seed peas contained a significantly higher RS_3 (97.6 ± 2.2 $g.kg^{-1}$) but lower total starch (310.1 ± 9.5 $g.kg^{-1}$) content. Its resistant/total starch proportion was 31.01 ± 0.95%. For all pea genotypes, a significant negative correlation was obtained (Fig. 4). The correlation coefficient value was –0.9557. These results are consistent with the observations of other authors who have documented a simultaneous decrease of amylopectin and total starch [37].

Long ago (1865), Johann Gregor Mendel discovered the fact that the wild-type pea had smooth seeds and was homozygous for the dominant *R* (rugosus) allele. Mutants contain the recessive allele *r* which causes wrinkling of the pea seeds. Smooth seeds are homozygous for *RR* or *Rr*, while wrinkled seeds are only homozygous for *rr*. Up until more than 100 years ago, it was recognized that the rr mutants had modified starch, therefore changes in osmotic pressure occurred. At the present time it

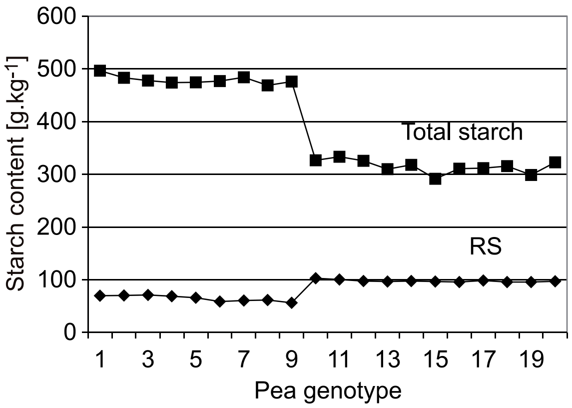

Fig. 4. Relationship between RS_3 and total starch content in 20 peas genotypes [g.kg^{-1} dwb].

is recognized that mutation at the *R* locus causes a loss of enzyme activity of the starch-branching isoenzyme SBEIIb which preferentially branches amylopectin [38]. Mutation at this locus results in a decrease in amylopectin and starch content. Mutants are comprised of high amylose, almost only of B-type granules and have wrinkled seeds. Changes in starch composition are manifested in physical and chemical properties - solubility, swelling, gelatinization, retrogradation and digestibility of starch [39-41].

Pea is very suitable natural source of health-promoting RS_3 starch, especially the wrinkled seed pea. The highest content was identified in the Ctirad, Dinara, Royal Salute and Elkan cultivars.

High RS_3 was also estimated in lentil cultivars, mainly in accessions L.C.M.B.12/58 and L.C.M.B.1941/68/58. Considering all legumes, the highest total starch content was found in lentil. Similar results for total starch have also been described by other authors [42]. JENKINS et al. [9] described the health-supporting effects of lentils, similar to those ascribed to resistant starch.

It is clear, from Fig. 3, that the kidney bean has a lower RS_3 content when compared to the pea or lentil, but similar to the faba bean and chickpea. The highest content of resistant starch was identified in the Ultima, Fabia, 545/9747/2 and 331/97 456/2 cultivars.

Of the faba bean cultivars, more resistant starch was found in the Alfréd and also, the Omar cultivars.

There were no difference in resistant and total starch between the Kabuli, Desi and Intermediate types of chickpea. The highest RS_3 levels were found in Alfa, CM-7-1/85, BG 004233, PK 51 814 and 88193.

Soybeans differ from the other evaluated legumes in resistant as well as total starch content. Total starch levels were very low, less than 100 g.kg^{-1} groats. Resistant starch levels were negligible, occurring below the limit of assay kit sensitivity. Soybean seeds contain mostly proteins and lipids.

Commercial products

Ten marketed commercial products were evaluated. For quality control, we excluded products from crops which have been identified as containing high resistant starch levels (buckwheat, rye and wheat). It can be seen in Tab. 1, that the amount of resistant starch in breads, crackers, slices and biscuits was within the limits of 2.3–8.0 g.kg^{-1} of product dry weight. The highest RS_3 content was found in wheat-rye expanded graham bread (producer Celpo, Breziny pri Slatine, Slovakia). In ad-

Tab. 1. Content of resistant starch RS_3 in commercial products [g.kg^{-1} dwb].

Product	Producer	Soluble starch	RS_3	Total starch
Graham crakers with buckwheat	ERI, Skalica, Slovakia	564.6 ± 9.1	5.9 ± 0.3	570.9 ± 8.4
Extruded slices	Pragosoja, Vidovice, Czech Republic	758.3 ± 4.5	3.8 ± 0.3	762.1 ± 4.8
Buckwheat sticks	PD Vlára, Nemšová, Slovakia	754.4 ± 7.7	4.9 ± 0.4	759.3 ± 8.2
Buckwheat sponge biscuit	Pečivárne, Liptovský Hrádok, Slovakia	436.0 ± 4.4	4.6 ± 0.4	436.2 ± 4.9
Extruded bread	PD Vlára, Nemšová, Slovakia	769.2 ± 3.9	5.3 ± 0.3	774.6 ± 4.2
Graham pastries	Extrudo Bečice, Týn n. Vltavou, Czech Republic	782.7 ± 6.9	4.3 ± 0.7	787.0 ± 6.2
Expanded bread	Celpo, Breziny pri Slatine, Slovakia	676.1 ± 1.9	8.0 ± 0.4	684.1 ± 2.3
Biscuits with 4 cereals (wheat, barley,oats, rye)	Opavia-LU, Praha, Czech Republic	476.3 ± 4.4	2.3 ± 0.3	478.6 ± 4.8
Wheat bread standard	Veľkopek, Piešťany, Slovakia	731.8 ± 4.6	21.5 ± 0.4	753.3 ± 4.2
Wheat-rye bread standard	Veľkopek, Piešťany, Slovakia	743.4 ± 5.2	26.7 ± 0.3	770.2 ± 6.1

dition, two breads with a weight of 2 kg (Veľkopek, Piešťany, Slovakia) were analyzed. In wheat and wheat-rye, dry weights of 21.5 and 26.7 $g.kg^{-1}$ respectively, were identified. The recommended daily dose of resistant starch in man is 3.2 g [43]. The latter quantity can be found in 150 g of wheat bread or in 120 g of wheat-rye bread sold on the market.

CONCLUSIONS

Significant differences in retrograded resistant starch were demonstrated within individual plant-crops and their cultivars. On the basis of the obtained results, 9 crops were identified as being very suitable natural sources of RS_3 resistant starch and at the same time, whose cultivation is possible in our climatic conditions. They are: rye, triticale, wheat, buckwheat, chickpea, pea, lentil, kidney bean and faba bean. The latter crops have higher starch amylose levels and are therefore, more capable of retrogradation and RS_3 formation. They represent a natural source of health-promoting starch for the production of functional foods.

Based on the legal cultivars registered in the Slovak Republic in 2005, we recommend the following cultivars: triticale Kendo, Pinokio, Presto and Tricolor; rye Dankowskie Nove and Esprit; wheat Boka; buckwheat Pyra; chickpea Alfa; kidney bean Fabia and Ultima; pea Elkan, Gloriosa and Zázrak z Kelvedonu; lentil Renka, and faba bean Omar.

The previously listed legumes are appropriate sources of health-promoting starch due to the suitable conditions for RS_3 formation during routine preparation by cooking or baking for approximately one hour. In addition, the Elkan, Gloriosa and Zázrak z Kelvedonu pea cultivars can be recommended for the freezing process of peas. The aforementioned cultivars are more able to form RS_3 in comparison with other cultivars.

In the case of cereals and pseudocereals, there are certain limitations. During experimental baking (20 min at 230 °C) we discovered that baking times of under 1 hour resulted in a lower amount of RS_3 formation in comparison to what the crop is able to form. We recommend the use of rye, triticale, buckwheat and wheat for baking products with weights of around two kilograms due to the longer baking times required. A particularly suitable sources of RS_3 is Boka wheat which also has a good baking quality. The ideal solution is the addition of rye or triticale flour to wheat flour for the production of functional foods from natural sources.

In order to obtain the health-promoting benefits of resistant starch, it is necessary that the individual possess a healthy colonic micro-flora (e. g. unaffected by antibiotic therapy).

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