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Effect of different cooking conditions on resistant starch and estimated glycemic index of macaroni

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ABSTRACT

Resistant starch is a type of carbohydrate that is slowly digested in the small intestine and fermented in the large intestine. Studies have been conducted to investigate the effects of different cooking methods (boiling, steaming, microwave, stir-frying, and deep frying), cooling and freezing on the quality of macaroni prepared with wheat flour and other resistant combinations starch sources. In this study, the *in vitro* digestibility of macaroni was determined and the glycemic index was estimated. Research results showed that cooking methods (boiling, steaming, microwave, stir-frying, and deep frying) reduced the resistant starch content of macaroni from 3.37 to 66.66%; however, cooling and freezing significantly increased the resistant starch content of macaroni from 6.88 to 24.19% and 9.85 to 37.28%, respectively. Macaroni prepared with the addition of flour/starch containing high levels of resistant starch exhibited a significantly lower estimated glycemic index (44.53–47.10) than the control sample using100% wheat flour (49.31).

1. INTRODUCTION

Resistant starch (RS) is defined as the total amount of starch and starch breakdown products that escape digestion in the small intestine of healthy people [1]. Because it is not digested in the small intestine, RS does not raise blood glucose and improve glycemic control. RS also has many other health benefits such as lowering cholesterol, increasing satiety, preventing constipation, and chronic diseases, including diabetes, colon cancer, and cases of obesity. During food processing, heat and humidity may be involved in the destruction of RS1 and RS2; however, RS3 can be formed. The microbiota ferments RS1, RS2, and RS3 in the large intestine [1,2]. Bacterial mass increases and production of short-chain fatty acids, providing many benefits to human health [2]. Besides, RS4 is esterified, etherified, or crosslinked with chemicals to reduce its digestibility. RS5 is a lipid component complex with amylose to form a helical structure and is formed under controlled conditions. RS5 is a polysaccharide composed of water-soluble linear poly α-1,4-D-glucan, which can be degraded by α-amylases [3]. The poly α-1,4-D-glucans have the potential to promote the formation of short-chain fatty acids, mainly butyric acid, in the colon and as nutritional supplements for the prevention of colorectal cancer [4]. RS2 is natural starch granules of selected plant origin, such as potatoes and green bananas. The shape or structure of the starch granules protected RS2 from digestion. The compact structure limits the accessibility of digestive enzymes and explains the resistant nature of RS2 [2,5]. The process of cooking and freezing makes the starches non-granular and crystallizes, becoming resistant to digestive enzymes; this is RS3 form. RS3 is a retrograded starch produced when gelatinization is followed by a long period of refrigeration [4,5]. The majority of RS3 results from heat treatment, mainly in the gelatinization and retrogradation stages [5].

RS formation during the processing of starchy foods is influenced by factors such as water content, temperature, and additional additives [6]. RS formation is dependent on amylose/amylopectin ratio, interaction with starch proteins, amylose lipid complexes, and starch retrogradation [7]. Starch tends to hydrate when cooked in water and become readily digestible rapidly. During cooling, the starch undergoes retrogradation, making the starches more crystalline and increasing their resistance to digestive enzymes [8]. Fuentes-Zaragoza *et al*. [8] reported that these degraded starch molecules are present in cooked cereals and cold potatoes. This is also why the glycemic index of cold cooked potatoes is lower than that of hot cooked potatoes [9]. Usually, starches tend to hydrate and become readily digestible when they are cooked. However, the starch undergoes a reverse degradation process that makes the starch more crystalline and increases its resistance to digestive enzymes when they are cooled.

The effect of heat-moisture treatment on RS content of pearl millet starch was studied by Sharma *et al*. [10]. They found that high humidity (30%) caused voids in starch granules and that RS content increased with this treatment. Chiu and Stewart [11] conducted studies on the influence of rice variety and cooking methods on the RS content

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of white rice. They reported that the lowest RS content was found in cooled short-grain rice cooked in a pressure cooker, while the highest levels of RS analyzed were obtained from cooled long-grain rice cooked in a conventional rice cooker. The glycemic indices did not show a significant differences between these samples and it has little effect on the postprandial glycemic response.

The National Institute for Health Metrics and Evaluation (2017) has forecast diabetes as one of the seven leading causes of death and disease in Vietnam by 2030 [12]. Therefore, researching and processing delicious and nutritious dishes with low energy value are a concern in Vietnam. The question is whether cooking methods can change the RS content of macaroni. In particular, the relationship between digestion rate and RS content of macaroni cooked by different methods is still not fully understood, especially for macaroni made from partial flour replacement with some common flour/starch-containing resistant starch, which is a common and abundant source of ingredients in Vietnam. This paper focuses on the influence of different cooking methods on macaroni's RS content and other estimates of the glycemic index (eGI) value of these products, which is a promising preparation for developing low-calorie and good-serving products in a developed society with increasing rates of obesity and diabetes.

2. MATERIALS AND METHODS

2.1. Materials

Mung bean, black bean, green banana, and purple sweets potatoes flours were prepared according to the previous studies [13,14]. All powders were stored in sealed PE bags and stored at room temperature (25°C) for further experiments. Potato starch (VINH THUAN Production Trading Import-Export Co., Ltd., Vietnam) was purchased at the local supermarket.

2.2. Effect of Cooking Methods on RS Content in Macaroni

Macaroni products were prepared by mixing all the ingredients into two formulas according to the specified ratio [Table 1]. The control sample M0 in this study was formulated with whole wheat flour and the sample M1 was prepared by replacing a part of wheat flour with high RS flours.

The resistant starch in all ingredients was measured as described by Thuy *et al*. [14]. They were all placed in equipment, stirred for 5 min, and then incubated for 30 min. After tempering time, macaroni was created by extruding the dough through a PHILIPS Pasta Maker [Figure 1]. The contents of salt, egg, water, and xanthan gum were fixed for both formulations.

Table 1: Two formulas of macaroni.

Ingredients	M0 (g)	M0 (%)	M1 (g)	M1 (%)
Mung bean powder	0	0	16	3.76
Green banana	0	0	16	3.76
Black turtle bean	0	0	16	3.76
Purple sweet potatoes	0	0	16	3.76
Potato starch	50	11.74	40	9.39
Wheat flour	200	46.97	146	34.29
Semolina	40	9.39	40	9.39
Salt	2	0.47	2	0.47
Egg	65	15.27	65	15.27
Water	63	14.8	63	14.8
Xanthan gum	5.8	1.36	5.8	1.36
Total	425.8	100	425.8	100

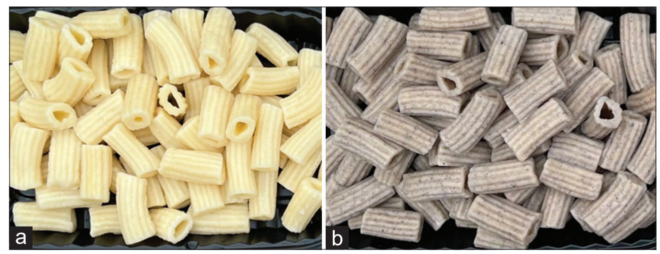

Figure 1: Macaroni–Control sample M0 (a) and supplemental sample of flour/starch source containing resistant starch M1 (b).

2.3. Cooking Methods

Five cooking methods such as boiling, steaming, stir-frying, frying, and microwave heating were applied to cook the macaroni. The cooling and freezing processes were also conducted.

Boiling: macaroni (50 g) was placed in 200 mL boiling water and boiled for 180, 210, 240, and 270 s.

Steaming: Macaroni (50g) was spread out evenly in trays with a thickness of about 2 mm and steamed at 100°C for 300, 600, 900, and 1200 s.

Stir-frying: Macaroni (50 g) was boiled in 200 mL boiling water for 150s and then cooled in running water for 30 s. Then, the cooked macaroni was done in a pan (180°C) with 20 mL soybean oil for 30, 60, and 90 s.

Deep-frying: Macaroni (50 g) was prepared as stir-frying before deep-fried in soybean oil (400 mL) in an electric frying pan (IESKIMOS, China) at 140, 160, and 180°C for 60 s.

Microwave-cooking: Macaroni (50 g) was put in a glass bowl with 200 mL boiling water and microwaved for 120, 150, and 180 s in a microwave oven (600 W, LG, Japan).

Cooling/Freezing: Boiled macaroni (50 g) was cooled at 4 ± 1°C and −10 ± 1°C for 12, 24, 36, and 48 h in a refrigerator and freezer (Sharp, Japan).

2.4. Determination of *In Vitro* Starch Digestibility and Estimated Glycemic Index (eGI)

In vitro starch digestion was determined as described by Englyst *et al*. [15] with slight modification by Chung *et al*. [16]. Cooked macaroni (under selected conditions by various cooking methods) is freeze-dried at −50°C and 0.045 mBar. The dried sample (containing 100 mg of starch and dry weight basis) was mixed with water (2 mL) in a 50 mL polypropylene centrifuge tube which was hydrolyzed by 1 mL of enzyme solution [pancreatic α-amylase and amyloglucosidase (AMG)] in a water bath (37°C, Memmert, Germany), shaking speed of 120 rpm. The released glucose content the during hydrolysis was determined using the K-GLUC kit (Megazyme, Ireland). Classification of starch-based on the rate of hydrolysis includes rapidly digestible starch (RDS, digested within 20 min), slowly digestible starch (SDS, digestible starch in the range of 20–120 min), and resistant starch (RS, indigestible starch after 120 min). The calculation of eGI of macaroni was carried out as described by Goni *et al*. [17]. Starch digestibility was expressed as the percentage of TS hydrolyzed at different time points. The first-order kinetics were applied for describe the *in vitro* starch digestibility (Eq. 1).

$$C_t = C_\infty \left(1 - e^{-kt}\right) \quad (1)$$

where, C_t is the percentage of starch hydrolyzed at time t (min), C_∞ is the equilibrium percentage of starch hydrolyzed after final time and k is the first-order rate constant.

The area under the hydrolysis curve (AUC) was calculated using the following Eq. 2.

$$AUC = C_\infty (t_f - t_o) - \left(\frac{C_\infty}{k}\right)\left[1 - e^{\left(-k\left(t_f - t_o\right)\right)}\right] \quad (2)$$

where, C_∞ is the equilibrium percentage of starch hydrolyzed after final time, t_f is the final time, t_0 is the initial time, and k is the first-order rate constant.

The hydrolysis index (HI) was calculated by dividing the AUC of each sample by the AUC of the reference sample (white bread). eGI was then calculated using the equation of Goni *et al*. [17] (Eq. 3).

$$eGI = 39,7 + 0,548 HI \quad (3)$$

2.5. Resistant Starch Analysis

RS tests were adapted for each matrix using the Association of Analytical Chemists [18] with some modifications [19]. The absorbance was measured at 510 nm against the reagent blank with a spectrophotometer (Beckman coulter DU 800).

2.6. Amylose Content Analysis

Amylose content of samples will be determined by spectrophotometer at wavelength 620 nm [20]. The best linear model selected was expressed by Eq. 4 (R^2 = 96%).

$$\text{Amylose content (\%)} = (5.83539 + 1.47157 \times \ln(\text{Abs620}))^2 \quad (4)$$

2.7. Moisture Content Analysis

The water content is determined by removing moisture by drying a certain mass of the sample at 105°C to constant mass and then by measuring the weight loss [18].

2.8. Statistical Analysis

Statistical analysis was performed using Statgraphics Centurion XV software (USA). At least three replications were performed for each treatment. To compare the mean values, an ANOVA test was used. Fisher's least significant difference (LSD) procedure was used to discriminate among the means of the variables at the 95% ($P \leq 0.05$) confidence limit.

3. RESULTS AND DISCUSSION

3.1. The RS and Amylose Content of Macaroni

RS content in the macaroni increased with an increase in the percentage of high RS starchy flour supplemented [Table 2]. A significant difference ($P < 0.05$) in the RS content between macaroni was shown. Amylose content has been shown to correlate with the RS of starchy food positively [14].

Table 2: The RS and amylose content of macaroni.

Formulation coded	RS content (%)	Amylose content (%)
M0	11.40±0.06	16.59±0.16
M1	18.62±0.38	17.71±0.08

Values are expressed as the mean±SD

3.2. Effects of Various Cooking Methods on Moisture, Amylose, and RS Content of Pasta

The obtained results shown that cooking conditions have the potential of decreasing and increasing the amount of RS [Table 3].

3.2.1. Effect of Boiling, Steaming, and Microwaving on Moisture, Amylose, and RS Content of Macaroni

As indicated in the table, the RS content decreased significantly with the time of boiling and steaming. When food is heated in the presence of water, starch granules absorb moisture, swell, and gelatinize, and amylose breaks down and washes out of solution [9,21] and a higher degree of gelatinization with longer heating time [19], which could explain the decrease in amylose and RS content along with the increase in moisture in boiled and steamed macaroni. The results of RS content of microwaved macaroni were comparatively higher than other cooking methods. At 180 s, the RS content was 25.61% (DW). A similar trend was found by Tian *et al.* [19], where the RS content of microwaved was higher than other samples prepared with different methods. However, the RS content of macaroni decreased after microwave cooking in comparison to uncooked sample, it may be due to gelatinization, increasing the digestibility of starches in the macaroni. The shorter time of cooking, a higher amount of RS was retained [22]. By this cooking method, the amylose content decreased with an increase in cooking time, but moisture content almost was similar to boiling. Heating occurred quickly and interaction between microwave radiation and starch resulted in the rearrangement of intramolecular structures during gelatinization, therefore, altering the water absorption capacity, solubility, swelling ability, and gelatinization characteristics [23]. Bilbao-Sáinz *et al.* [24] reported that gelatinization of microwaved starch granules in a short time required more energy than the conductive heat process, which may aid explain the high RS content of microwaved which showed a more compact structure [19]. Narwojsz *et al*. [25] also reported that microwave heating helps to maintain the RS content of potatoes at a higher level than steaming and boiling at different cooking times. High amylose content limits swelling compared to native starches with lower amylose content, positioning modified starch as a food matrix stabilizer. In addition, cooking starches with high amylose content result in a low degree of gelatinization while leaving high levels of RS in the form of dietary fiber [26].

3.2.2. Effect of Frying Conditions on the Moisture Content, Amylose Content, and RS of Macaroni

It was observed that the RS content decreased with processing time. The highest RS content remained after 30 s of stir-frying (25.49% DW) and decreased when stir-frying for 90 s (21.89% DW). However, these data obtained were lower than uncooked macaroni. Our results are quite similar to the previous studies [19,27], it may be due to the free fatty acid in the oil may form an inclusion complex with the amylose helix thereby decreasing the content of amylose available for retrogradation and subsequent RS formation [6]. However, Dhital *et al*. [28] found that the RS content of stir-fried increased from 7 to 12%. The amylose and moisture content also showed significantly different reductions with increasing cooking time.

Table 3: The moisture, amylose, and RS content of macaroni by various cooking methods (boiling, steaming, microwave heating, and fryings).

Cooking methods	Cooking time (seconds)	Moisture content (%)	Amylose content (% DW)	RS content (% DW)
Uncooked	0	30.96	25.65	26.97
Boiling	180	65.08[a]	44.01[b]	12.43[a]
	210	65.40[a]	42.25[ab]	14.28[c]
	240	66.46[b]	41.56[a]	13.86[b]
	270	69.70[c]	52.54[c]	14.29[c]
Steaming	300	35.35[a]	23.87[a]	12.03[b]
	600	36.09[b]	24.19[b]	11.77[a]
	900	40.22[c]	25.59[c]	12.35[bc]
	1200	43.84[d]	25.93[c]	13.16[c]
Microwave	120	61.09[a]	40.94[b]	26.06[ab]
	150	62.25[b]	41.22[b]	26.70[b]
	180	62.56[b]	39.08[a]	25.61[a]
Stir-frying	30	55.75[c]	34.19[c]	25.49[c]
	60	42.96[b]	25.18[b]	20.51[a]
	90	41.47[a]	22.96[a]	21.89[b]
Deep-frying	140°C	8.87[c]	16.96[c]	9.89[c]
	160°C	2.52[b]	15.19[b]	9.22[b]
	180°C	0.85[a]	13.42[a]	8.99[a]

*Values with different letters in the same column (for each cooking method) are significantly different at $P<0.05$

Deep frying of macaroni had a notable effect on RS cooked at different temperatures. At 140°C to 160°C of deep-frying, the RS content was 9.89% to 8.99% (DW). It was evident that as the frying temperature increased, the RS content decreased. The color and flavor of the also changed due to Maillard reactions between sugar and amino acids present in the macaroni [28]. Similar observations were obtained by Yadav *et al.* [27] in deep frying potatoes, where RS content of the potatoes decreased significantly after deep frying. An increase in time and temperature of deep frying resulted in a decrease in RS content because the absence of water in fried samples inhibits the crystallization of amylose chains. The RS and amylose contents of samples were lower than stir-fried macaroni, the moisture content decreased significantly with an increase in frying temperature. Vaidya and Sheth [6] also found that frying showed a decrease in RS content. Frying reduced the RS content of potatoes and sweet potatoes by 28.0% and 32.0%, respectively, with deep frying showing a more pronounced effect. Mahadevamma and Tharanthan [7] also showed decrease in RS content on frying. The difference in RS content in stir-fried and deep-fried can be explained by their different moisture content [19] because water is required in the forming amylose-lipid complex [29]. Besides RS, the frying conditions also affect the moisture and amylose content of the pasta products. The moisture content of the decreases significantly with the stir-frying time and the frying oil temperature. In addition, the lack of water in the fried samples also inhibited the crystallization of the amylose chain, leading to a decrease in the RS content after frying [27]. As a result, stir-fried macaroni often has a higher RS content than deep-fried (as mentioned above). However, several studies have shown that pasta heat treatments such as steaming, frying, or cooking lead to an increase in RS content [28]. These differences are probably due to the source of RS used, equipments, and processing techniques.

3.2.3. Effect of Cooling and Freezing on the Moisture Content, Amylose Content, and RS of Macaroni

Macaroni was boiled at the appropriate time (210 s) and further cooled at 4 ± 1°C and −10 ± 2°C with a controlled time of up to 48 h. The obtained results showed that the RS content of macaroni gradually increased from 14.28% to 17.73% and 19.60% DW (increase of 1.24 and 1.37 times) after 48 h of cooling and freezing, respectively [Table 4]. These results are quite consistent with some previous reports [30], which showed that the RS content of bread increased significantly during the first 3–4 days of storage at 4–−20°C. However, they showed that cooling offers a higher RS content than freezing. The difference between researches may be due to the difference of the type of food and food moisture. Borczak *et al*. [31] also concluded that freezing at −18°C increased RS content of wheat flour rolls in the first 3 days, and these contents did not change furthermore. Gelatinized and fragmented amylose molecules undergo molecular reassociation or degradation during cryopreservation, resulting in increased RS content [32]. Several previous study showed that one type of RS is formed when foods are cooled after cooking, this process is called starch retrogradation [33]. It occurs when some starches lose their original structure due to heating or cooking. If these starches are later cooled, a new structure is formed [34], its structure is resistant to digestion and leads to health benefits. Cooling the white rice has increased the resistant starch content, cooked white rice cooled for 24 h at 4°C followed by reheating reduces the glycemic response compared with freshly cooked white rice [35]. If these steps were done, the RS can be increased in common foods, such as rice, bread, and pasta.

The amylose content of macaroni increases gradually with the loss of moisture due to dehydration with cooling/freezing time. When cooled, the recrystallization or molecular degradation of amylose occurs rapidly because the amylose is a straight linear chain, so it is easy to form hydrogen bonds between them and this process is slower for amylopectin due to its branched nature inhibits recrystallization to some extent [33,34].

3.3. *In Vitro* Starch Digestibility Characteristics and Estimated Glycemic Index of Macaroni under Different Cooking Methods

As mentioned above, cooking affected on the RS of macaroni. Starch is the main component in the processing of products and is directly related to the glycemic index or glycemic response after eating [36].

The starch digestibility properties of pasta products cooked under different techniques were evaluated. The results of starch properties of macaroni under different cooking conditions through starch digestion are presented in Table 5.

The rapidly digestible starch (RDS) proportion of macaroni ranged from 14.83±0.39% in microwave-heated sample to 19.55 ± 0.70% in boiled sample. RDS defines as a type of starch that is digested rapidly in the small intestine. In addition, RDS is closely related to the rapid rise in postprandial blood glucose levels and simultaneously represents the hydrolysis of starch chains at or near the grain surface [33]. Chemically, it is measured by the content of glucose released or percentage of starch hydrolyzed during the first 20 min of enzymatic hydrolysis [33]. With this short time interval, hydrolytic enzymes cannot penetrate the grain interior because diffusion must occur before hydrolysis [37]. The RDS content in macaroni under different cooking conditions reflects the reversible effect between surface characteristics and the degree of molecular order at the grain surface [38]. The morphology of cooked by different methods also clearly showed the difference in the outer and inner layer structure, which also affected the digestibility of noodle products [19].

The percentage of SDS content, which is digested more slowly, varies slightly between cooking methods, with the highest content in the microwave product (56.51 ± 1.15%) and the lowest in the boiled (53.81 ± 0.86%) and deep-fried (53.76 ± 0.99%) products. The stability of the crystal granules and the arrangement of the double helices in the crystal region (both of which limit the access of amylolytic enzymes to the glycosidic bonds) directly affect the SDS content in the sample. Moreover, SDS is regularly recommended by nutritionists to be used and it is considered as a dietary starch, which helps to control several diseases such as obesity, diabetes, cardiovascular, and some cancers [37].

Table 4: Effect of cooling/freezing on moisture, amylose (DW), and RS content (DW) of cooked macaroni.

Cooling/Freezing time (h)	Moisture content (%)	Amylose content (% DW)	RS content (% DW)
Cooling (4±1°C)			
0	65.40[b]	42.25[a]	14.28[a]
12	64.72[b]	42.51[a]	15.26[b]
24	63.96[ab]	47.08[b]	17.06[c]
36	63.34[ab]	55.63[c]	17.59[d]
48	62.71[a]	53.36[c]	17.73[d]
Freezing (−10±1°C)			
0	65.40[b]	42.25[a]	14.28[a]
12	65.25[b]	47.60[b]	15.68[b]
24	63.88[a]	48.59[c]	16.67[c]
36	63.57[a]	49.05[cd]	18.25[d]
48	63.01[a]	49.07[d]	19.60[e]

Values with different letters in the same column (for each cooking method) are significantly different at $P<0.05$

Table 5: The proportion types of starch in macaroni.

Cooking methods	RDS (%)	SDS (%)	RS (%)
Boiling	19.55±0.70*	53.81±0.86	26.86±1.21
Steaming	18.89±0.39	54.01±0.53	27.06±0.41
Microwave	14.83±0.39	56.51±1.15	28.73±0.71
Stir-frying	14.94±0.33	56.46±0.44	28.59±0.18
Deep-frying	16.90±0.73	53.76±0.99	29.32±0.90

*Values are expressed as mean±SD

A significant change in the proportion of RS content in macaroni prepared by different methods was also observed, with the highest in fried macaroni, followed by stir-fried, microwaved, steamed, and the lowest in the boiled products. Boiled and steamed samples were digested faster than others, followed by microwave-heated. The digestibility of sample after deep-frying and stir-frying is slower. The equilibrium percentage of starch hydrolyzed ($C\infty$) was between 17.21% (deep-frying method) and 22.69% (boiling method). Among them, steamed and boiled macaroni had the highest percentage of starch hydrolysis, similar to the study by Reed *et al*. [39]. Moreover, eGI value of the sample using 100% wheat flour (sample M0) boiled at 100°C for 3 min (eGI = 49.31) was higher than that of the selected sample (sample M1) after cooking in different methods, which similar with our previous result when incooporated starchy flour into noodle [40,41]. Partial replacement of wheat flour in noodle recipes with RS-containing ingredients helps limit the rapid rise in the glycemic index after eating.

The eGI values of macaroni were in order, boiled macaroni (47.10) > steamed macaroni (46.97) > microwaved macaroni (45.67) > stir-fried macaroni (45.19) > deep-fried macaroni (44.59). Hoover *et al*. [42] reported differences in starch digestibility due to the interplay of many factors, such as starch source, grain surface organization, amylose degradation, amylose-lipid complex, degree of crystallinity, polymorphic crystal form, and presence of other compound particles.

Tian *et al*. [19] also suggested that a small portion of starch coated and linked with protein or fat remained in cooked, forming RS1. On the other hand, the results also showed that starch and lipids in after boiling and steaming form a partial complex, namely, RS5. As reported by Bilbao-Sáinz *et al*. [24], gelatinization of starch granules cooked in a microwave (for a short time) requires more energy than samples heated in water, which may be related to the higher RS content. For stir-fried and deep-fried samples, starch can easily form amylose-lipid compounds (RS5) [43], as starch in can react with oil during cooking. As a result, more RS5 is formed during stir-frying and deep-frying the resulting in its lower digestibility. The results of Pearson correlation showed that the RS content of macaroni was negatively correlated with its digestion rate, that is, macaroni with higher RS content had a slower rate of digestion. The correlations between pasta product starches and eGI were evaluated with the result that RDS was negatively correlated with RS and SDS ($r = -0.774$ and $r = -0.620$, respectively). RDS was positively correlated with eGI ($r = 0.722$), where eGI increased as RDS increased or/and SDS decreased ($r = -0.810$). These results are consistent with some previous studies [44,45].

The results of this study initially showed a significant change in RS content in macaroni processed by different methods. GI values of macaroni were also evaluated. However, the GI value is also affected by the different preparation methods and composition of the test food (46). This is also a limitation of the study when it was published mainly with macaroni products. Another limitation of the study is that the different cooking methods used by restaurants today also affect the GI value of this food [46]. Therefore, more in-depth research is needed in terms of household processing and serving in restaurants, first, possibly locally with macaroni and further with other foods that resistant starch is used in food recipes. Further, *in vivo* testing should be done to provide more valuable information regarding the effects in a living organism.

4. CONCLUSIONS

Different cooking methods (boiling, steaming, microwave, stir-frying, and deep-frying) reduced the RS content of macaroni, the lowest RS loss is found for the microwave cooking (3.37%) and the highest is shown for the deep-frying (66.66%), while cooling and freezing tended to increase the RS content from 1.24 to 1.37 time with appropriate time, respectively. Microwave heating and cooling/freezing were suitable methods to maintain and improve the RS content in macaroni products. The obtained values also showed a positive effect of the ingredients containing resistant starch added in the macaroni formulation on the glycemic index of the product. Macaroni with RS-rich flours/starches has a significantly lower estimated glycemic index (eGI) than sample made with only wheat flour. The low glycemic index of is closely related to the SDS and the RS in the product. Due to its potential health benefits, appropriate cooking methods should be preferred to increase RS content of macaroni and other food products; however, the effectiveness of this product needs further *in vivo* study.

5. AUTHORS' CONTRIBUTIONS

Conceptualization, methodology, and supervision: Nguyen Minh Thuy; formal analysis, investigation, writing-original draft preparation, writing-review, and editing: Nguyen Minh Thuy and Ngo Van Tai. All authors have read and agreed to the published version of the manuscript.

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7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

All data generated and analyzed are included within this research article.

10. PUBLISHER'S NOTE

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