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Research article

DEVELOPMENT OF RESISTANT STARCH TYPE-5 AND ITS UTILIZATION IN COOKIE-PREPARATION

Betül Oskaybaş-Emlek[1]✉, Ayşe Özbey[1], Kevser Kahraman[2]

[1]Department of Food Engineering, Engineering Faculty, Niğde Ömer Halisdemir University, Niğde, Türkiye
[2] Department of Nanotechnology Engineering, Engineering Faculty, Abdullah Gül University, Kayseri, Türkiye
✉Corresponding author: Betül Oskaybaş-Emlek, Department of Food Engineering, Engineering Faculty, Niğde Ömer Halisdemir University,
✉betuloskaybas@ohu.edu.tr
ORCID Number: 0000-0002-0238-8948

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Abstract
The objective of this study was the production of resistant starch type-5 (RS-5), its characterization, and utilization in cookie making. In first part of the study, the effects of starch-fatty acid complex formation (RS-5) between tapioca starch and lauric acid on the structure, digestibility, thermal and morphological properties of tapioca starch were investigated. X-ray diffraction revealed that the RS-5 had a V-type crystalline pattern. FT-IR analysis showed that a distinctive peak at 2846 cm^{-1} was only observed in RS-5. The resistant starch (RS) content of native starch increased from 22.76% to 28.02% with RS-5 formation. In the second part of the study, the RS-5 was added as a replacement for wheat flour with 10%, 20%, and 30% compared to control sample made with 100% wheat flour in cookie-making. The effects of RS-5 replacement of cookie samples on some physicochemical, estimated glycemic index (eGI) value, physical, and hardness properties were determined. Compared to control cookie, the cookie samples included RS-5 had lower hardness value, higher spread ratio. The eGI value of cookie samples was slightly decreased with the replacement with RS-5. The results demonstrated that the RS-5 has good potential for developing softer cookie with no adverse impact on eGI value.

1. Introduction

Starch, a storage compound in plants, consists of two polysaccharides which are amylose and amylopectin (Li *et al.*, 2021). Three categories of starch are distinguished by their digestibility characteristics: Rapidly Digestible Starch (RDS), Slowly Digestible Starch (SDS), and Resistant Starch (RS) (Oskaybaş-Emlek *et al.*, 2022a). RS consists of complex carbohydrates, which can resist digestion and absorption in the small intestine (Stewart and Zimmer, 2017). It is classified into five groups according to structural properties. RS-1 is physically inaccessible starch, RS-2 is granular starch with B- or C-XRD pattern, retrograded starches are RS-3, and modified starches with chemical agents are RS-4. In addition, RS-5 is formed the starch-fatty acid complex (Stewart and Zimmer, 2017).

The starch-fatty acid complex can be naturally present in starch or formed during starch gelatinization in the presence of lipids (Garcia *et al.*, 2016). It was known that the apolar chains of fatty acid molecules can interact with the hydrophobic cavity of amylose through hydrophobic interaction resulting in starch-fatty acid complexes. Many types of starch have been used in the formation of amylose lipid complexes, such as corn (Sun *et al.*, 2021), buckwheat (Oskaybaş-Emlek *et al.*, 2022b), wheat (S. Wang *et al.*, 2016), rice, potato and tapioca starch (Ramos-Villacob *et al.*). Besides, based on previous studies about starch-fatty acid complex, very little information concerning the tapioca starch-lauric acid complex is available (Ramos-Villacob *et al.*). Additionally, this RS-5 was formed with tapioca starch and lauric acid at the temperature below the gelatinization temperature (60°C) (Ramos-Villacob *et al.*). There are various studies in the literature that the starch-lipid complex formed at a temperature below (Chang *et al.*, 2013; D'Silva *et al.*, 2011) and also above the pasting temperature (Ai *et al.*, 2013; Reddy *et al.*, 2018). However, Seo *et al.* (2015) reported that the complex formation at a relatively low temperature leads to less-ordered complexes. The reaction temperature of complex formation affects the digestibility properties of starch-fatty acid complex samples (Oskaybaş-Emlek *et al.*, 2022a). Therefore, the reaction temperature used in the production of the complex may affect the beneficial effects of the complex on human health.

Resistant starch is known as beneficial functions in human physiology, such as reducing the risk of diabetes (Rojhani *et al.*, 2022), preventing the development of colon cancer, having beneficial effect on blood lipid levels (Sharma *et al.*, 2008), and protecting against the obesity (Han *et al.*, 2023). Additionally, Zheng *et al.* (2020) reported that the higher RS-5 level may have contributed to the significant reduction in body weight and healing in serum-lipid profiles and liver function of rats under a high-fat diet. In addition to the health benefits of RS, it has many techno-functional properties, giving advantages to food products. Milašinović Šeremešić *et al.* (2013) reported that the adding RS to various confectionery products led to an improvement in softness structure. RSs can be used to give the final product the desired texture and appropriate crispness in food products. Besides, RSs improve the flavor, color, and aroma of some foods (Milašinović Šeremešić *et al.*, 2013). The existence of starch-fatty acid complexes (RS-5) in starch supports the development of new starch-based products with appropriate structure and functionality (Sengupta *et al.*, 2023). Adding starch- fatty acid complexes (RS-5) to food products decreases starch's retrogradation, i.e., it delays the staling process (Luo *et al.*, 2020; Sengupta *et al.*, 2023). The RSs have many advantages in terms of the physiology of humans and technological aspects of the food industry. Therefore, RSs practically can be utilized in many bakery products.

Cookies are among the most widely consumed snack foods due to their low cost, convenience, and long shelf life (Rojhani *et al.*, 2022). There are many studies in the literature on cookie enrichment with dietary fiber and RS (Aparicio-Saguilán *et al.*, 2007; Giuberti *et al.*, 2017; Kahraman *et al.*, 2019; Rojhani *et al.*, 2022). However, RS is a more preferred alternative compared to dietary fiber due to the cookies with higher dietary fiber content having a coarser and denser structure (Rojhani *et al.*, 2022). Additionally, RS has no negative effects on the sensory properties of food such as texture, appearances because it has small particle size, bland flavor, and white color (Rojhani *et al.*, 2022). However, in the literature, there are very little information about the utilization of RS-5 in cookie-making (X.-H. Chen *et al.*, 2022; Xiaohan Chen and Wang, 2024; Garzóan *et al.*, 2003). On the other hand, to the best of our knowledge, there is no information regarding the use of the RS-5 complexation between lauric acid and tapioca starch in the cookie production.

In this study, the RS-5 was formed (>60°C) by the complexation between tapioca starch and lauric acid and the impact of complex formation on the morphological, FT-IR structure, XRD pattern, and digestibility properties of tapioca starch was examined. Additionally, another purpose of this study was to investigate some physico-chemical (pH, a_w, color), physical (spread ratio) and texture characteristics of cookies prepared using RS-5 as a replacement for 10%, 20% and 30% of wheat flour as compared to a control made with 100% wheat flour.

2. Materials and methods

2.1. Materials

Lauric acid (C12:0), pancreatin (P7545) and pepsin (P7000) were bought from Sigma Aldrich (Sigma-Aldrich, ABD). Tapioca starch and sodium acetate were purchased from Tito Gıda (Turkey) and Merck Millipore Corporation (Germany), respectively. Glucose oxidase-peroxidase (GOPOD) reagent was supplied from Megazyme International (Ireland). Ingredients used in cookie production were purchased from a local store in Nigde, Turkey.

2.2. Methods

2.2.1. Production of Resistant Starch Type-5 (RS-5)

The RS-5 was produced using a Rapid Visco-Analyzer (RVA 4500, Perten Instruments, Sydney, Australia) according to S. Wang *et al.* (2017) with slight modifications. For this purpose, 3.5 g (14% moisture, db) and lauric acid (350 mg) were mixed with deionized water to make a total weight of 28 g. To produce the RS-5 between tapioca starch and lauric acid, the STD 1 profile provided with RVA was performed. The obtained RS-5 was freeze-dried, then grounded with a laboratory mill (<212 µm). The RS-5 sample and native tapioca starch were stored (+4°C) until further analyses.

2.2.2. Characterization of RS-5

The complex index value (CI) of RS-5 was determined according to method described by S. Wang *et al*. (2016).

The rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) content of native tapioca starch and RS-5 were determined according to Oskaybaş-Emlek *et al.* (2022a).

The XRD patterns of native tapioca starch and RS-5 were obtained using Bruker AXS D8 X-Ray diffractometer (Karlsruhe, Germany) with CuKα radiation (λ = 0.15405 nm) operating at a voltage current of 40 kV and 30 mA. Data were collected from 2θ of 5°-40°.

The structural properties of native tapioca starch and RS-5 were investigated with Thermo Nicolet Avatar 370 Fourier Transformed Infrared Spectrometer (FT-IR) (Madison, WI, USA). The wavenumber range was determined as 4000–400 cm−1 and scanning time was 32 for each sample

The morphological properties of native tapioca starch and RS-5 was analyzed using a scanning electron microscope (Zeiss Sigma 300 Field Emission SEM, Oberkochen, Germany). Prior the analysis, the samples coated with a thin layer of gold. Images were taken at 15.00 kV.

Thermogravimetric analysis was conducted with a STA TG-DSC/DTA PT1600 (Linseis, Germany) The operation was performed from 20 °C to 700 °C at a heating rate of 10 °C/min under nitrogen.

2.2.3. Preparation of cookies

In the cookie production, RS-5 was replaced with wheat flour at different ratios (0, 10, 20 and 30%). The cookies were prepared according to the method of Oskaybaş-Emlek *et al.* (2021) with slight modifications. The water content of cookie dough preparation was 30 mL. After baking, the cookies were cooled to room temperature (23°C), then they were packaged in polyethylene bags, until used.

2.2.4. Cookie characterization

The estimated in-vitro glycemic index value (eGI) of cookie samples were determined according to method described by Kahraman *et al.* (2019).

The physical properties of the baked cookies were determined were thickness, diameter and spread ratio (Diameter/Thickness). The thickness and diameter of cookies were measured using digital caliper.

The color properties of cookies were determined using colorimeter (Konica Minolta CR 400, Japan) (Oskaybaş-Emlek *et al.*, 2021). The results were expressed in terms of L* (lightness/ darkness), α* (redness/greenness) and b* (yellowness/blueness)

The Texture Analyzer (TA.XT Plus Texture Analyzer, Godalming, UK) using with a three-point bending jig was used to determine the textural properties of cookies in terms of break strength. The analysis was performed after baking 24 h. Test parameters were determined as test speed of 3.0 mm/s and strain value of 10.0%.

The water activity values (a_w) of cookie samples were measured with a water activity meter (Novasina Labswift, Switzerland). Scanning electron microscope system (Jeol JSM-6480LV, Japan) was used to examine morphological of dried powder before and after enzyme treatment.

The pH value of grounded cookie samples (10% (w/v) suspension in distilled water) was determined with a pH meter (Hanna Instruments, Malaysia).

2.3. Data analysis

Independent t-test ($p_{value} < 0.05$) was performed to compare the data of native tapioca starch and RS-5 samples. Data of cookie samples were analyzed using IBM SPSS Statistics version 24.0 (SPSS Inc., Chicago, IL) with analysis of variance with Duncan's test multiple comparison ($p_{value} < 0.05$) between treatments.

3. Results and discussions

3.1. Some characteristic properties of RS-5

Some characteristic properties of RS-5 are shown in Table 1. The complex index value (CI) is determined by the ratio of free amylose components in starch-lauric acid complex to iodine complexation compared to untreated starch. In other words, this value means the percentage complex formation of tapioca starch with lauric acid (D'Silva *et al.*, 2011). The CI value of RS-5 was 90.16%.

Table 1. The properties of native tapioca starch and Resistant starch type-5 (RS-5)

Parameters	Samples	
	Native starch	**RS-5**
Complex index value (CI%)	-	90.16
Rapidly digestible starch (%)	43.03*	40.28*
Slowly digestible starch (%)	33.20	30.71
Resistant starch (%)	22.76*	28.02*

*: Means within each row are significantly different according to independent t-test ($p_{value} < 0.05$).

The rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) content of native tapioca starch and RS-5 samples are displayed in Table 1. The RDS, SDS and RS content of native tapioca starch were 43.03%, 33.20% and 22.76%, respectively. In literature, there are various studies on the RDS, SDS, and RS content of tapioca starch. For example, Corgneau *et al.* (2019) reported that the RDS, SDS, and RS content of native tapioca starch were 19.6%, 37.8% and 42.7%, respectively. Mei *et al.* (2015) showed that the RDS, SDS and RS content of native tapioca starch were approximately 85%, 8%, and 5%, respectively. Different digestibility properties can be influenced by several factors, including the ratio of amylose to amylopectin, the degree of crystallinity of the starch, the structure of the starch molecule, the length of the amylopectin chains, the particle size, and the protein content of the starch, among others (Xu Chen *et al.*, 2017; Farooq *et al.*, 2018; Kim *et al.*, 2017; Oskaybaş-Emlek *et al.*, 2022a). The complex formation of tapioca starch with lauric acid led to decrease in RDS (40.28%) and SDS (30.71%). However, complex formation resulted in increase in RS (28.02%) content.

This situation was revealed that the complex formation between tapioca starch and lauric acid led to increase in starch's resistance to digestible enzymes. Similarly, Ramos-Villacob *et al.* (2024) observed that digestible properties of native tapioca starch and tapioca starch-lauric acid complex. They reported that RDS, SDS and RS content of native tapioca starch were 72.98%, 17.06%, and 9.96%, respectively. In addition to these, they observed that RDS, SDS and RS content of tapioca starch-lauric acid complex ranged from 36.48 to 75.61%; 10.87 to 44.61%; 11.00 to 18.90%. The starch-fatty acid complex may form an insoluble film on the starch granule. This structure has the potential to block the transportation of enzymes into the granule, thereby enhancing resistance to digestion (Oskaybaş-Emlek *et al.*, 2022a). According to Y.-S. Wang *et al.* (2020), the complex formation led to develop a more structure form, which enhanced its decrease in susceptibility of starch to digestion enzymes.

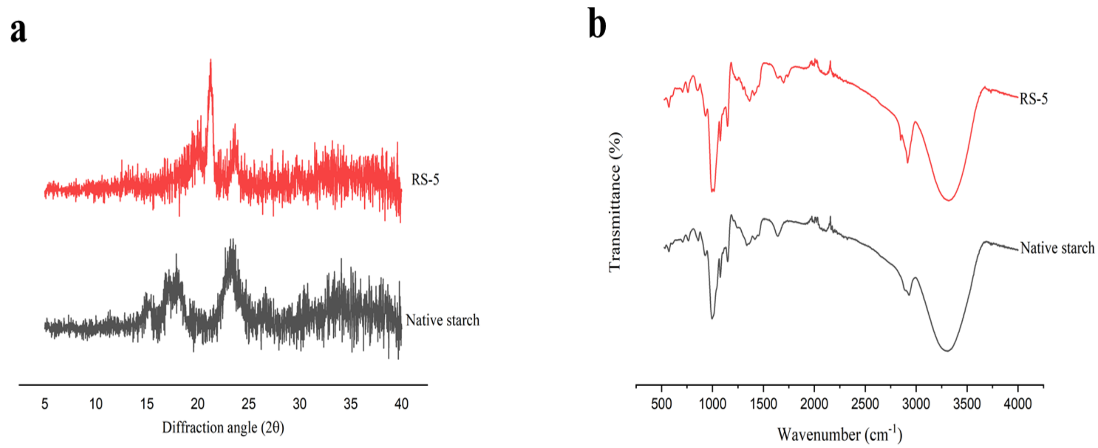

Figure 1.a- X-ray diffraction (XRD) patterns and **b-** FT-IR spectra of starch samples. RS-5; Resistant starch type-5.

The X-Ray diffraction patterns of native tapioca starch and RS-5 are shown in Figure 1a. Native tapioca starch had a typical A-type crystalline pattern with peaks at 15°, 17°,18°, ve 23° (Xia *et al.*, 2015). The complex formation of native tapioca starch with lauric acid led to emergence of a distinct diffraction at 19.9°, which can be attributed to the starch-fatty acid complex (S. Wang *et al.*, 2016). In addition, the diffraction angle at 21.6° found in the starch-lipid complex pattern belongs to the crystal form of free fatty acids (S. Wang *et al.*, 2016).

FTIR spectroscopy was utilized to verify the change in the chemical structures of tapioca starch molecules resulting from resistant starch formation. The FTIR spectra of native tapioca starch and RS-5 sample are shown in Figure 1b. In the spectrum of native tapioca starch and RS-5, the peaks at 3300 and 1600 cm^{-1} correspond to OH groups, i.e, these peaks were related to water molecule found in starch (Oskaybaş-Emlek *et al.*, 2022b; Yun *et al.*, 2020). Additionally, in two starch samples (native starch and RS-5), there was a strong absorption band at 2900 cm^{-1}, which were attributed to stretching vibration of C-H bond (Mendes *et al.*, 2021). The peaks at between 1340 and 1400 cm^{-1} was attributed to stretching of OH groups (Oskaybaş-Emlek *et al.*, 2021). The region between the 800 and 1200 cm^{-1} bands is associated with intermolecular hydrogen bonds of the double helices of amylose and amylopectin or amylose-amylopectin complexes (Oskaybaş-Emlek *et al.*, 2022b). Compared to native starch, RS-5 had a absorption band at 2848 cm^{-1}. This band was related to asymmetric stretching vibration of CH_3 and CH_2 of fatty acids. In literature, the band at 2848 was observed in starch-fatty acid complexes compared to native starch (Sun *et al.*, 2021; S. Wang *et al.*, 2016).

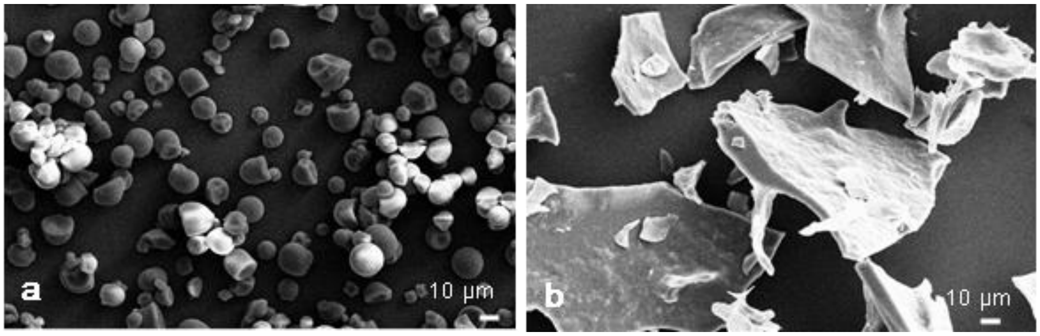

Figure 2. Scanning electronic microscope (SEM) images of **a-** Native tapioca starch **b-**RS-5. Magnification 1.0 Kx. RS-5; Resistant starch type-5.

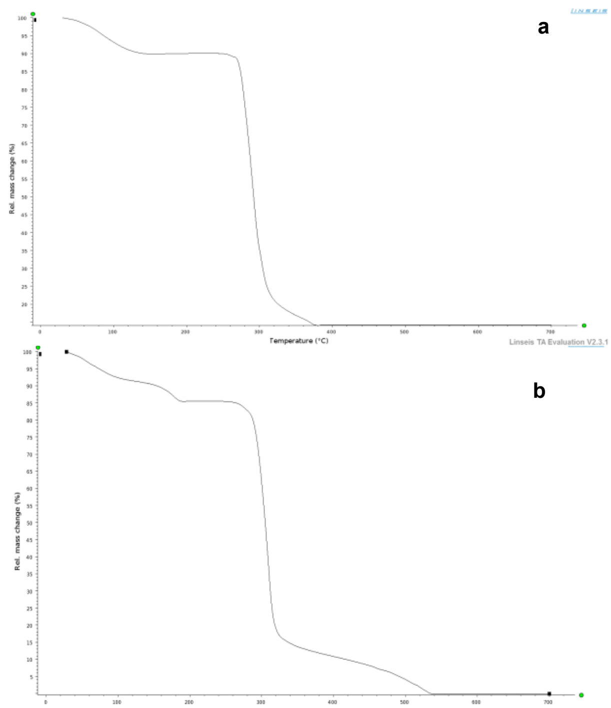

Fig. 3. TGA curves of starch samples **a-** Native tapioca starch **b-**RS-5. RS-5; Resistant starch type-5.

The scanning electron microscopy of the native tapioca starch and RS-5 are given in Figure 2. The SEM of tapioca starch (Figure.2a) exhibited a spherical granules with smooth surface. Similar observation was reported by (Xia *et al.*, 2015). However, the granule structure of tapioca starch was obviously changed by RS-5 formation between native tapioca starch and lauric acid. During RS-5 production, the starch granules swelled and ruptured, therefore the original structure was destroyed. After RS-5 formation, starch granule was disintegrated and aggregated, and thus, it became irregular. In Figure. 2b, microcrystals in the shape of protruding spherulites formed on the particle surface as a result of the RS-5 formation (Wu *et al.*, 2022). Additionally, RS-5 have larger granule size compared to native tapioca starch, as also reported by Z. Chen *et al.* (2024).

The TGA curves of native tapioca starch and RS-5 are presented in Figure 3. According to Figure 3, the thermal decomposition process of starch showed three stages in the temperature range of 20-700°C. The first stage occurred between 20-120°C and 20-200°C for native tapioca starch and RS-5, respectively, which was related to the weight loss of water evaporation (Rodríguez-Huezo *et al.*, 2018). The second stage for native tapioca starch and RS-5 occurred at approximately 120-350°C and 200-350°C, respectively. In this stage, the thermal weight loss was associated with starch degradation and degradation of guest molecules (Liu *et al.*, 2022). The weight loss in the last stage appeared at 320°C-380°C for tapioca starch, while this temperature reached 540°C for RS-5. The weight loss was ascribed to the decomposition of the ash (Liu *et al.*, 2022). According to TGA curves (Figure 3), the temperature value required for the degradation of RS-5 was approximately 140°C higher than the native starch. This situation revealed that the thermal stability of starch improved with the formation of the RS-5 sample.

3.2. Some characteristic properties of cookie samples

Table 2 shows the characteristics of cookie samples. The highest aw value belonged to cookie with 30% RS-5 replacement level (0.48) (p_{value} <0.05). This increase was related to the higher water binding capacity of the RS-5 than that of wheat flour (Water absorption capacity data of wheat flour and starch samples were not shown). The a_w value is crucial in assessing food safety regarding microbial risks. The aw values suitable for the development of most bacteria, yeasts and molds are in the range of 1-0.87, 0.91-0.87 and 0.87-0.65, respectively (Oskaybaş-Emlek *et al*., 2021).

Table 2. The properties of cookie samples

Parameters	**Cookie samples**			
	Control	**10% RS-5**	**20% RS-5**	**30% RS-5**
a_w	0.41^b	0.44^{ab}	0.47^{ab}	0.48^a
pH	6.77^a	6.69^b	6.62^c	6.61^c
$L*$	78.44^a	78.10^a	77.81^a	76.84^a
$a*$	-0.24^a	-0.56^b	-0.65^c	-0.93^d
$b*$	27.29^a	24.19^b	25.00^b	22.28^c
eGI	149.75^a	147.62^a	144.41^a	143.37^a
Diameter (mm)	59.75^a	56.5^b	55.5^c	55.25^c
Thickness (mm)	7.75^a	7.75^a	7.00^b	7.25^{ab}
Spread ratio	7.72^{ab}	7.30^b	7.96^a	7.63^{ab}
Hardness (g)	12363.87^a	8184.52^b	7048.89^c	4724.42^d

$^{a-d}$ Mean values in the same row with different superscript letters differ significantly (p_{value} < 0.05)
RS-5; Resistant starch type-5, aw; water activity, eGI; estimated glycemic index value.

According to these a_w values, the risk of microbial growth in the cookie samples produced within this study is very low. The pH value of cookie samples was significantly decreased by replacement of RS-5. The cookie samples including 30% RS-5 had the lowest pH value (6.61). The decrease in pH may be due to the fact that RS-5 contained lauric acid, i.e, the increase in replacement of RS-5 led to increase in lauric acid content in cookie.

The L*, α* and b* values were determined within the scope of the color properties of cookie samples. The L* value represents lightness-darkness and that value is in the range of 100-0 (Bawa *et al.*, 2020). The L* value of control cookie was 78.44, and it slightly decreased to 76.84 with RS-5 replacement levels ($p_{value} > 0.05$). According to Table 2, the RS-5 replacement led to less yellow (lower b*) and more green-looking cookie (− α*: greenness) ($p_{value} < 0.05$).

The estimated glycemic index (eGI) value of control cookie sample was 149.75. The eGI value of cookies decreased with the replacement of wheat flour with RS-5. However, there were no significant difference among the eGI of cookie samples ($p_{value} > 0.05$). It may be due to the fact that the replacement level is not sufficient to decrease in eGI. These unexpected results could be due to the fact that the replacement ratio with wheat flour is not sufficient. Additionally, the eGI value of cookie made with the replacement of wheat flour with RS-5 may not have significantly decreased because of properties of wheat flour such as amylose content, relative crystallinity value (Kunyanee and Luangsakul, 2020), protein content (Jimenez-Pulido *et al*., 2022).

The cookie with 20% RS-5 replacement level had the highest spread ratio. However, there were no significant difference among spread ratio values of control sample, cookie with 20% and 30% RS-5 replacement level ($p_{value} > 0.05$). According to Mudgil *et al.* (2017), the spread ratio is related to the quality of the cookie. A higher spread ratio is an indicator of a desirable cookie.

The hardness value of cookie samples ranged from 4724.42 g to 12362.87 g ($p_{value} < 0.05$). The hardness value decreased with an increase in the RS-5 replacement level. The decrease in cookie hardness with the increase in RS-5 can be associated with a decrease in protein content. In other words, the increase in the RS-5 replacement level reduced the protein from wheat flour in cookie samples (Khouryieh and Aramouni, 2012). Similarly, Bae *et al.* (2013), observed that the hardness value of cookie samples diminished with increasing resistant starch content.

4. Conclusions

The complex formation of tapioca starch with lauric acid greatly affected the digestibility, crystallinity, and thermal properties of starch. V-type crystalline pattern was formed between tapioca starch and lauric acid. With complex formation, RS content of starch (from 22.75% to 42.70%) and thermal stability of starch enhanced. Morphological properties of tapioca starch were significantly affected with RS-5 formation. Additionally, the cookie samples with RS-5 replacement had a softer texture compared to the control sample, which is among the desired cookie characteristics. The eGI of cookie was not affected by the replacement of wheat flour with RS-5. This situation revealed that there are many factors affecting eGI value in addition to RS content. This study has also shown that the RS-5 produced with buckwheat starch and lauric acid is an applicable option that the increase in spread ratio. The results were especially good at the 20% substitution level when evaluating the general physical and textural characteristics of the cookie samples with RS-5 substitution.

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