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BUCKWHEAT STARCH-MYRISTIC ACID COMPLEX FORMATION: EFFECT OF REACTION TEMPERATURE AND MYRISTIC ACID CONCENTRATION ON DIGESTIBILITY PROPERTIES

Betül Oskaybaş Emlek[1], Ayşe Özbey[1], Levent Yurdaer Aydemir[2], Kevser Kahraman[3*]

[1]Dept. of Food Eng., Niğde Ömer Halisdemir University, Niğde, Türkiye
[2] Dept. of Food Eng., Adana Alparslan Türkeş Science and Technology University, Adana, Türkiye
[3]Dept. of Nanotechnology Eng., Abdullah Gül University, Kayseri, Türkiye

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ABSTRACT

This study aimed to investigate the effect of different reaction conditions on the digestibility properties of buckwheat starch-myristic acid complex samples produced using different myristic acid concentrations and different reaction temperatures. Response Surface Methodology was used to investigate the effect of reaction temperature (60-90°C) and fatty acid concentrations (0.1-0.8 mmoL/g) on digestibility properties. Resistant starch (RS) contents of samples increased with an increase in reaction temperature. The reaction temperature affected the rapidly digestible starch (RDS) and slowly digestible starch (SDS) content of samples. The highest RS content (32.57%) was obtained using 0.45 mmoL/g myristic acid at 90°C. The F, p (<0.05), and R^2 values indicated that the selected models were significant for the digestibility properties of samples. The complex formation of buckwheat starch with myristic acid seems promising to increase the RS content. Buckwheat appears to have the potential as an RS source, although the studies are quite new yet.

Keywords: buckwheat starch, myristic acid, resistant starch, starch-lipid complex.

KARABUĞDAY NİŞASTASI-MİRİSTİK ASİT KOMPLEKS OLUŞUMU: REAKSİYON SICAKLIĞI VE MİRİSTİK ASİT KONSANTRASYONUNUN SİNDİRİLEBİLİRLİK ÖZELLİKLERİ ÜZERİNE ETKİSİ

ÖZ

Bu çalışma, farklı miristik asit konsantrasyonları ve farklı reaksiyon sıcaklıkları kullanılarak üretilen karabuğday nişastası-miristik asit kompleksi örneklerinin sindirilebilirlik özellikleri üzerine farklı reaksiyon koşullarının etkisini araştırmayı amaçlamıştır. Reaksiyon sıcaklığının (60-90°C) ve yağ asidi konsantrasyonlarının (0.1-0.8 mmoL/g) sindirilebilirlik özellikleri üzerindeki etkisini araştırmak için Yanıt Yüzey Metodolojisi kullanılmıştır. Örneklerin enzime dirençli nişasta (EDN) içerikleri reaksiyon sıcaklığındaki artışla artmıştır. Reaksiyon sıcaklığı, örneklerin hızlı ve yavaş sindirilebilir

* *Corresponding Author* / Yazışmalardan sorumlu yazar
✉: kevser.kahraman@agu.edu.tr ✆: (+90) 352 224 8800 🖷: (+90) 352 338 8828

Betül Oskaybaş Emlek; ORCID no: 0000-0002-0238-8948
Ayşe Özbey; ORCID no: 0000-0003-3210-4077
Levent Yurdaer Aydemir; ORCID no: 0000-0003-0372-1172
Kevser Kahraman; ORCID no: 0000-0002-2786-3944

nişasta içeriklerini etkilemiştir. En yüksek EDN içeriği (%32.57) 90°C'de 0.45 mmoL/g miristik asit kullanılarak üretilen örnekte elde edilmiştir. F, p (<0.05) ve R^2 değerleri seçilen modellerin numunelerin sindirilebilirlik özellikleri için önemli olduğunu göstermiştir. Karabuğday nişastasının miristik asit ile kompleks oluşturması, EDN içeriğini artırma konusunda umut verici görünmektedir. Karabuğday, çalışmalar henüz oldukça yeni olmasına rağmen, EDN kaynağı olarak önemli bir potansiyele sahip görünmektedir.
Anahtar kelimeler: Karabuğday nişastası, miristik asit, dirençli nişasta, nişasta-lipit kompleksi

INTRODUCTION

Starch can be considered one of the significant components of cereal-based foods (Sun et al., 2019) and is used as one of the important energy-providing components. Starch provides more than 50% of the human energy intake needs in western countries. Additionally, in developing countries, this ratio has increased to approximately 90% (Sun et al., 2019). Therefore, the digestibility properties of starch have attracted attention to human nutrition.

Based on enzymatic digestibility, starch can be classified into three major groups; rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). RDS is digested in the small intestine in 20 minutes and has a high glycemic blood glucose level. SDS is digested between 20 min and 120 min and has a lower glycemic response than that of RDS. On the other hand, RS is not digested in the small intestine; instead, it is fermented in the large intestine by microbes to generate short-chain fatty acids (Asare et al., 2021; Englyst et al., 1992; Liu et al., 2019; Okumus et al., 2018). Therefore, the digestibility properties of starch have a direct effect on human health. Many health advantages of RS have been recorded, including a decrease in plasma cholesterol and lipids and an increase in mineral absorption (Chung et al., 2009; Kahraman et al., 2019). In addition, it helps reduce the post-meal glycemic response and prevent colon cancer (Asare et al., 2021). Due to its limited digestion and comparable physiological features, RS is regarded as a component of dietary fiber (Nugent, 2005; Sharma et al., 2008).

The five types of RS in foods are categorized as; RS1-physically entrapped in the cellular matrix (such as whole ground grain, legumes); RS2-some type of native starch granules (such as green banana, potato); RS3-retrograded starch (e.g. cooked potato, pasta); RS4-chemically modified starches (such as starch esters, cross-linked starches); RS5-amylose-lipid complexes (Hasjim et al., 2010; Kahraman et al., 2019; Kahraman et al., 2015; Okumus et al., 2018).

Due to the previously described health advantages of RS, the production of amylose-lipid complexes has recently attracted the attention of researchers worldwide. Amylose-lipid complex formation can be present naturally in native starch or can be formed by the interaction between amylose and lipid during food storage or processing (Wang et al., 2017). The hydrocarbon chain of lipids (small non-polar molecules, hydrophobic domains of amphiphilic molecules such as fatty acids, monoglycerides, and surfactants) interacts with the helical cavity of amylose with hydrophobic interactions to form an amylose-lipid complex (Hasjim et al., 2013; Okumus et al., 2018; Tang and Copeland, 2007). In addition to this, amylopectin can partially participate in amylose-lipid complex formation due to having branched structure (Hasjim et al., 2013). It is well known, that amylose can form V-amylose inclusion complexes with lipids, and thusly, these complexes can hinder starch swelling and enzymatic digestion (Chung et al., 2009; Sun et al., 2019). According to the literature, there are several reaction parameters (reaction pH; reaction time and temperature; chain length of amylose and fatty acid; type, saturation degree and content of fatty acid; starch source) affecting the amylose-lipid complex formation, hence, these reaction conditions affect the digestibility profile (Asare et al., 2021; Chao et al., 2020; Chen et al., 2017; Hasjim et al., 2013; Kawai et al., 2012; Li et al., 2019; Marinopoulou et al., 2016; Raza et al., 2021; Seo et al., 2015; Sun et al., 2021; Yassaroh et al., 2021).

Only the effects of high hydrostatic pressure, γ-irradiation, heat-moisture treatment and annealing on physicochemical, digestibility, and functional qualities of buckwheat starch have been studied in the literature (Dar et al., 2018; Goel et al., 2020; Liu et al., 2015; Liu et al., 2016; Xiao et al., 2017). However, to the best of our knowledge, there is no information in the literature on the effect of buckwheat starch-myristic acid complex formation on the digestibility properties of buckwheat starch.

In this study, starch-lipid complexes were produced from buckwheat starch and myristic acid at varying reaction temperatures and fatty acid concentrations using response surface methodology (RSM). The objective of this study was to understand how reaction temperature and myristic acid concentration influence the digestibility properties of buckwheat starch-myristic acid complexes.

MATERIALS AND METHODS

Materials

Buckwheat flour was purchased from Ingro Food Informatics Marketing (Karaman, Turkiye). Prior to starch production, the flour sample was passed through a 212 mμ sieve. Myristic acid (C14:0), pancreatin (P7545) and pepsin (P7000) were supplied from Sigma Aldrich Chemical Co., Ltd. (St. Louis, Mo., USA). Amyloglucosidase (3300 U/mL) and glucose oxidase-peroxidase (GOPOD) reagent were obtained from Megazyme International (Ireland). Sodium acetate was purchased from Merck Millipore Corporation (Germany). All other chemicals used in this study were of analytical grade.

Buckwheat starch isolation and characterization

The buckwheat starch was isolated according to the method defined by Gao et al. (2016) with some modifications, as described in our previous study (Emlek et al., 2022). Moisture, ash, total lipid, protein (N: 6.25) and starch contents of buckwheat starch were determined according to the AACCI Methods 44-15A, 08-01, 30-25.01, 46-11A and 76-13.01, respectively (AACCI, 2000).

Starch-fatty acid complex formation

Response surface methodology (RSM) was used to investigate the effect of reaction conditions on the digestibility properties of buckwheat starch-myristic acid complexes The reaction temperature and myristic acid concentration were chosen as two independent variables. The RDS, SDS, and RS content of the starch myristic acid complexes were selected as the dependent variable. The reaction temperature values and myristic acid concentration values were determined using complex index results of our preliminary study, as described by our previous study. According to our previous study, it was decided that the reaction temperature should have been around the starch gelatinization temperature in order to complex the formation between buckwheat starch and fatty acid (Emlek et al., 2022). In the preliminary study, fatty acid concentration values were used as 0.5 and 2 mmoL/g. However, the effect of the difference in these values on the complex index value was not significant. Therefore, it was decided that the fatty acid values should start from lower than 0.5 mmoL/g (Emlek et al., 2022). The reaction temperature values were 60, 67.5, 75, 82.5, and 90°C and myristic acid concentrations were 0.1, 0.275, 0.45, 0.625, and 0.8 mmoL/g. Fifteen reaction temperature and myristic acid concentration combinations were created with three points in the center (Table 1).

The starch-myristic acid complexes were prepared according to the method of Reddy et al. (2018) with some modifications (Emlek et al., 2022). For this purpose, buckwheat starch (10 g) and myristic acid were dispersed in 200 ml of the reaction solution. Chemlab buffer solution (pH 5, Zedelgem, Belgium) was used as the reaction solution. The reactions were carried out for 0.5 h by using a magnetic stirrer (400 rpm), which was equipped with a temperature controller (Heidolph Mei Tec Heater, Germany). After the reaction, the starch-myristic acid complexes were cooled to 25°C and centrifuged at 9000 x *g* for 10 min. The supernatant was discarded, while the precipitates were collected and washed with diethyl ether (450 mL) to remove free fatty acid in the precipitates. The samples were kept under a fume hood for 1 h to remove diethyl ether. Then, the complex

samples were freeze-dried, ground with a laboratory mill to pass a 212 μm sieve and stored at +4°C until further analysis.

Table 1. Rapidly digestible starch (RDS), slowly digestible starch (SDS) and Resistant starch (RS) contents of buckwheat starch-myristic acid complexes

Run	Myristic acid concentration (mmoL/g)	Reaction Temperature (°C)	RDS (%)	SDS (%)	RS (%)
Native buckwheat starch			54.03^{a}	26.97^{fg}	19.01^{g}
1	0.1	60	42.17^{cdef}	35.43^{bc}	22.40^{f}
2	0.45	60	39.41^{fg}	38.54^{ab}	22.05^{f}
3	0.8	60	42.49^{bcde}	31.94^{de}	25.57^{de}
4	0.275	67.5	38.67^{gh}	37.99^{ab}	23.34^{ef}
5	0.625	67.5	36.49^{h}	39.24^{a}	24.26^{ef}
6	0.1	75	40.39^{defg}	33.85^{cd}	25.76^{de}
7	0.45	75	43.04^{bcde}	32.73^{cde}	24.23^{ef}
8	0.45	75	43.30^{bcd}	33.21^{cd}	23.50^{ef}
9	0.45	75	42.87^{bcde}	33.05^{cde}	24.08^{ef}
10	0.8	75	44.31^{bc}	31.41^{de}	24.28^{ef}
11	0.275	82.5	45.19^{b}	26.11^{g}	28.70^{bc}
12	0.625	82.5	42.44^{bcde}	25.96^{g}	31.60^{a}
13	0.1	90	41.56^{cdefg}	31.27^{de}	27.17^{cd}
14	0.45	90	40.17^{efg}	27.25^{fg}	32.57^{a}
15	0.8	90	39.47^{fg}	29.71^{ef}	30.82^{ab}

* Means with different letters within each column are significantly different ($p < 0.05$)
RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch

Starch digestion

The rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) content of starch-fatty acid complexes were determined based on the method of Englyst et al. (1992) and according to the procedure described by Kahraman et al. (2019) with slight modification. Briefly, starch samples (100 mg) were mixed with 2 mL of distilled water in 50 mL polypropylene centrifuge tubes. The suspensions were cooked in a boiling water bath for 20 min with vortexing every four minutes. Then, 2 mL of pepsin/0.05 M hydrochloric acid solution (5 mg/mL) was added and the tubes were incubated in a shaking water bath at 37°C for 30 min. Thereafter, sodium acetate buffer (4 mL, 0.5 M, pH 5.2) was added to tubes. Starch digestion was initiated by adding one milliliter of freshly prepared enzyme solution (0.104 g of pancreatin and 14.26 U amyloglucosidase) in a shaking water bath at 37°C. The aliquots (100 μL) of the hydrolysate were taken at different times (20 and 120 min) and mixed with 1 mL of ethanol to stop further digestion. Afterward, these solutions were centrifuged (800 × g, 10 min), and hydrolyzed glucose content of the supernatant was measured with glucose oxidase-peroxidase (GOPOD) reagent by spectrophotometer at 510 nm. The hydrolyzed glucose content at 20 and 120 min was labelled as G20 and G120, respectively. RDS, SDS, and RS content of the sample were calculated using formulas as follows:

$$RDS = G20\ X\ 0.9 \quad (1)$$

$$SDS = (G120 - G20)\ X\ 0.9 \quad (2)$$

$$RS = TS - RDS - SDS \quad (3)$$

where the TS means the total starch content of samples.

Statistical analysis

All of the results are reported as means of at least duplicate analyses. Data were analyzed by the IBM SPSS Statistics Trial Version 15.0 (SPSS Inc., Chicago, IL) with a one-way analysis of variance (ANOVA). When significant (P<0.05) differences were found, Duncan's test was used to determine the differences among means.

The response value was fitted by regression models using Design Expert Trial Version 11.0.4.0 (Stat-Ease, Minneapolis, MN, USA) to evaluate the relationship between RDS, SDS and RS (dependent variables) and reaction temperature and fatty acid concentration (independent variables). Based on two variables (reaction temperature and myristic acid concentration), the user-defined method ($\alpha = 1$) was applied to the matrix design of the experiments. Three-dimensional response surface plots and regression equations were also developed.

RESULTS AND DISCUSSION

Characterization of buckwheat starch

The total ash, protein, lipid and starch content of buckwheat starch were 0.09%, 0.34%, 0.47% and 92.80%, respectively. A similar result was observed by Liu et al. (2015).

In vitro digestibility of buckwheat starch-myristic acid complexes

The RDS, SDS and RS contents of native buckwheat starch and buckwheat starch-myristic acid complexes produced using various myristic acid concentrations and reaction temperatures are displayed in Table 1. The RDS, SDS and RS content of native buckwheat starch were 54.03, 26.97, and 19.01%, respectively. Our results were in agreement with those of Du et al. (2022), who determined differences in the digestibility of buckwheat starch from different cultivars. They reported that RDS, SDS and RS content of common buckwheat starches ranged from 43.64 to 62.62%; 26.22 to 37.09% and 9.54 to 22.75%, respectively. Furthermore, those values were observed between 49.3 -75.02%, 20.18- 37.38% and 4.80- 20.15%, respectively, for starch isolated from Tartary buckwheat cultivars (Du et al., 2022).

Table 1 shows that the RDS, SDS and RS content of buckwheat starch-myristic acid complexes varied from 36.49 to 45.19%, 25.96 to 39.24%, and 22.05 to 32.57%, respectively. The RDS content of all buckwheat starch-myristic acid complex samples was significantly lower compared to the native buckwheat starch, whereas the SDS+RS concentration was much higher. This indicated that buckwheat starch-myristic acid complexes might be more resistant to enzymatic digestion than native buckwheat starch. According to Sun et al. (2021), the RDS is partially converted to RS with complex formation, hence, complex samples become more resistant to digestion. Complex formation between starch and lipid resulted in a decrease in the swelling of granules during heating in excess water owing to the presence of an insoluble film on the granule surface. As a result, water transport into granules and enzyme accessibility to starch granules were inhibited (Ai et al., 2013; Hasjim et al., 2013; Okumus et al., 2018; Wang et al., 2016). Another reason for the increase in resistance to digestion may be the formation of organized structures which are resistant to starch hydrolysis during starch-lipid complex production (Wang et al., 2020). Our results were consistent with those previously reported by Ai et al. (2013), Sun et al. (2021) and Wang et al. (2016), who reported that the starch-lipid complex presented higher resistance to digestive enzymes than native starch.

In the case of buckwheat starch-myristic acid complexes produced at the same reaction temperature, the increase in myristic acid concentration generally caused an increase in RS content. The increase was significant for some samples (P <0.05). For example, at 90°C reaction temperature, increasing the myristic acid concentration from 0.1 to 0.45 and 0.8 mmoL/g significantly increased the RS content of the samples (P <0.05). This was related to an increase in amylose-lipid complexation with an increase in fatty acid concentration, in line with complex index (CI) results, as stated by our previous study (Emlek et al., 2022). The CI% value of buckwheat

starch-myristic acid complexes produced using 0.1, 0.45, and 0.8 mmoL/g at 90°C were 86.1%, 95,0%, and 94.6%, respectively. CI value is an indication of starch-lipid complex formation. Similar results were observed by Li et al. (2019). According to their findings, the CI and RS values of starch-palmitic acid complexes increased as the fatty acid concentration increased from 0 to 2%, and this was attributed to rising in amylose-lipid complex formation (Li et al., 2019). On the other hand, there is no correlation between CI and RS content for all samples. For instance, when the reaction temperature was 60°C, the difference between the RS content of the sample produced using 0.1 mmoL/g myristic acid (22.40%) and 0.45 mmoL/g myristic acid (22.05%) was not significant ($P > 0.05$). On the other hand, at 60°C, the increase in myristic acid concentration from 0.1 to 0.45 mmoL/g slightly led to a decrease in the RDS content of the sample from 42.17% to 39.41% and an increase in SDS content from 35.43% to 38.54%. The results partially agree with the CI value of samples as reported in our previous study (Emlek et al., 2022). The complex index value of the samples produced at 60°C in the presence of 0.45 mmoL/g myristic acid concentration was significantly higher than that of the one produced in the presence of 0.1 mmoL/g (Emlek et al., 2022). It seems that starch-lipid complex formation was one of the factors affecting the starch digestibility properties among many others. Similarly, Sun et al. (2022) showed that the RS content of high amylose maize starch-fatty acid complexes was not directly dependent on the complex index value and relative crystallinity of the samples. According to the previous findings, many factors such as amylose-amylopectin ratio, starch crystallinity, molecular structure and chain length of amylopectin, amylose-lipid complex formation, particle size, protein content, fatty acid structure, and fatty acid binding degree can affect starch digestibility properties (Chen et al., 2017; Farooq et al., 2018; Kim et al., 2017; Okumus et al., 2018; Oyeyinka et al., 2017; Sun et al., 2021; Sun et al., 2022).

At the same fatty acid concentration, the increase in the reaction temperature generally resulted in a significant ($P < 0.05$) increase in the RS content (Table 1). For example, at the same reaction temperature (67.5°C) the buckwheat starch-myristic acid complexes obtained using 0.275 and 0.625 mmoL/g myristic acid had 23.34% and 24.26% of RS content, respectively. And the increase in reaction temperature to 82.5°C led to a significant ($P < 0.05$) increase in RS content to 28.70% and 31.60%, respectively. Similar results were also found by Wang et al. (2020) with starch-lauric acid complexes produced at different reaction temperatures. According to them, the increase in temperature increased the amount of leached amylose, and thus more amylose-lipid complexes occurred. As indicated in our previous research, increasing the complexation temperature may enhance the mobility of starch chains, which can increase the interaction between starch and fatty acid and lead to the formation of more amylose-lipid complexes (Emlek et al., 2022). In addition to these, Hasjim et al. (2010) and Wang et al. (2020) reported that amylose-lipid complexes were more resistant to enzymatic digestion than only amylose. In the case of the buckwheat starch-myristic acid complexes produced using 0.45 mmoL/g myristic acid, increasing the reaction temperature from 60°C and 75°C to 90°C increased the RS content of the samples. On the other hand, the difference in CI value among these samples was not significant, as stated in the previous study (Emlek et al., 2022). Similarly, Sun et al. (2021) observed that the RS content of maize complexes with various fatty acids was not related to their CI value and crystallinity degree. Our findings indicate that there were many factors influencing starch digestibility properties.

The effect of myristic acid concentration and reaction temperature on the digestibility properties of buckwheat starch-myristic acid complexes

To describe the relationship between the independent variables (myristic acid concentration and temperature) and dependent variables (digestibility properties; RDS, SDS, and RS contents), the response values were fitted to third-order polynomial (cubic) regression models. ANOVA analyses results for the models are presented in Table 2.

Table 2. Analysis of variance (ANOVA) for the RSM design

Source of varience	Degrees of freedom	RDS (%)		SDS (%)		RS (%)	
		F	*P*	*F*	*P*	*F*	*P*
A (myristic acid concentration)	1	4.59	0.0988	1.90	0.2399	1.25	0.3256
B (Temperature)	1	34.68	0.0042*	377.34	<0.0001*	18.61	0.0125*
AB	1	1.29	0.3191	1.51	0.2862	0.1287	0.7379
A^2	1	3.32	0.1424	0.8872	0.3996	1.13	0.3481
B^2	1	3.28	0.1445	0.0745	0.7984	2.64	0.1793
A^2B	1	1.87	0.2431	56.42	0.0017*	5.86	0.0728
AB^2	1	6.49	0.0635	0.0060	0.9421	4.59	0.0987
A^3	1	9.50	0.0369*	6.31	0.0659	1.85	0.2453
B^3	1	24.80	0.0076*	125.81	0.0004*	1.40	0.3018
Model	9	6.10	0.0486*	69.69	0.0005*	9.17	0.0237*
Lack of fit	3	17.27	0.1746	40.23	0.1153	13.25	0.1986
R^2		0.9321		0.9937		0.9538	

*indicates the significant factors ($P < 0.05$)
RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch

In the case of the RDS, SDS and RS content of buckwheat starch-myristic acid complexes, the *P* values of the models were 0.0486, 0.0005 and 0.0237, respectively, indicating that the models were reliable. The coefficients of determination (R^2) of the models indicated that only 6.79%, 0.63%, and 4.62% of the total variation could not be explained by the models, respectively. The temperature (coded as B) had significant effects on the RDS, SDS and RS contents of the samples ($P = 0.0042$, $P < 0.0001$, and $P = 0.0125$, respectively). In addition, the cubic effect of temperature (coded as B^3) had a significant effect on the RDS and SDS content of the samples ($P = 0.0076$ and $P = 0.0004$, respectively). On the other hand, the fatty acid concentration (coded as A) did not significantly affect the RDS, SDS, and RS contents of buckwheat starch-myristic acid complex samples ($P > 0.05$). However, the cubic effect of myristic acid concentration (coded as A^3) had a significant effect on the RDS content of the samples ($P = 0.0369$). In addition to this, the interaction between the quadratic effect of fatty acid concentration (coded as A^2) and only the linear effect of temperature (coded as B) had a significant effect on the SDS content of the samples ($P = 0.0017$).

The analysis of variance for the final reduced models obtained by eliminating the nonsignificant terms identified were presented in Table 3. For RDS, SDS, and RS, the developed empirical models obtained from Table 3 are given in Eqs. (4)–(6). The A and B are the coded values of independent variables (myristic acid concentration and temperature, respectively).

$$RDS\ (\%) = 42.15 + 8.47B - 8.95B^3 \quad (4)$$

$$SDS\ (\%) = 32.77 - 16.24B + 4.05A^2B + 10.60B^3 \quad (5)$$

$$RS = 25.08 + 3.84B \quad (6)$$

Eqs. (4)–(6) are plotted as 3D response surface plots and shown in Figure 1a, 1b, and 1c, respectively. The figures represent the effects of different fatty acid concentrations and temperatures on RDS, SDS, and RS contents of buckwheat starch-myristic acid complexes respectively. The effect of the interaction between reaction temperature and myristic acid concentration on the digestibility properties of buckwheat starch-myristic acid complexes can be inferred from the Figures. The blue and red regions in Figures 1a, 1b and 1c indicate the lowest and the highest values, respectively. As can be seen in Figure 1a, the increase in reaction temperature from 66 to 84°C caused an increase

in the RDS content of the samples. According to Figure 1b, at the same myristic acid concentration, the increase in temperature generally led to a decrease in the SDS content of samples. In Figure 1c, it can be seen that RS formation was enhanced as the reaction temperature increased at the same concentration of myristic acid. On the other hand, the increase in the myristic acid concentration at the same temperature value did not cause a change in the SDS and RS contents of the samples (Figure 1b and 1c.).

Table 3. Analysis of variance (ANOVA) for the final reduced models

Source of varience	Degrees of freedom	RDS (%)		SDS (%)		RS (%)	
		F	*P*	*F*	*P*	*F*	*P*
A (myristic acid concentration)	1	-	-	-	-	-	-
B (Temperature)	1	14.87	0.0027*	119.63	<0.0001*	26.62	0.0002*
		-	-	-	-	-	-
AB	1	-	-	-	-	-	-
		-	-	-	-	-	-
A^2	1	-	-	-	-	-	-
B^2	1	-	-	-	-	-	-
A^2B	1	-	-	17.89	0.0017*	-	-
AB^2	1	-	-	-	-	-	-
		-	-	-	-	-	-
A^3	1	-	-	-	-	-	-
B^3	1	14.37	0.0030*	39.88	<0.0001*	-	-
		-	-			-	-
Model	9	7.49	0.0089*	63.37	<0.0001*	26.62	0.0002*
Lack of fit	3	32.84	0.1351	106.53	0.0751	24.81	0.1555
R^2		0.5765		0.9500		0.6893	

*indicates the significant factors ($P < 0.05$)
RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch

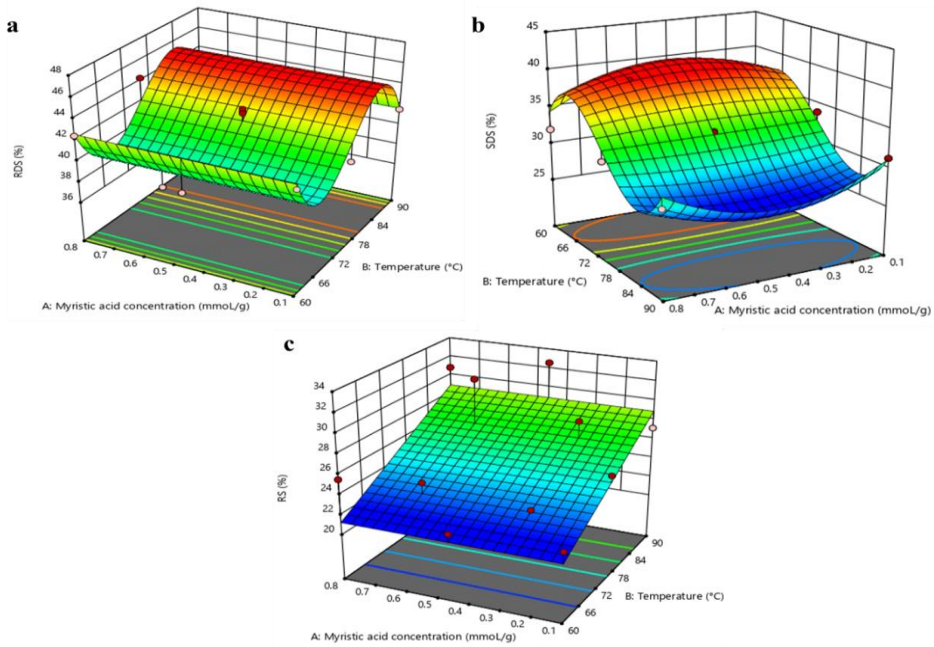

Figure 1. 3D response surface plots of a) RDS, b) SDS, and c) RS contents of buckwheat starch-myristic acid complexes. RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch.

CONCLUSIONS

This research revealed how the myristic acid concentration, as well as the reaction temperature, affect the buckwheat starch-myristic acid complex digestibility. Compared to native buckwheat starch, buckwheat starch-myristic acid complex formation resulted in an increase in RS content and a reduction in RDS content. Regarding the RS content of the buckwheat starch-myristic acid complexes, the reaction temperature was more effective than the myristic acid concentration. The RSM equations demonstrated that it may be possible to achieve high RS content using myristic acid concentration and temperature above 0.4 mmoL/g and 82°C, respectively. RS results of buckwheat starch-myristic acid complexes indicated that buckwheat starch-myristic acid complexes can be a potential RS5 source and can be used as an RS alternative in food formulations to produce healthy foods. Further studies are needed to produce buckwheat starch-myristic acid complexes with high RS content.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

AUTHOR CONTRIBUTIONS

Betül Oskaybaş Emlek: Conceptualization, Software, Validation, Formal analysis, Writing - Original Draft, Writing - Review & Editing, Visualization.

Ayşe Özbey, Conceptualization, Methodology, Validation, Writing - Review & Editing, Supervision, Visualization. Levent Yurdaer Aydemir, Conceptualization, Methodology, Validation, Writing - Review & Editing, Supervision, Visualization. Kevser Kahraman, Conceptualization, Methodology, Validation, Writing - Review & Editing, Supervision, Project administration, Visualization.

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