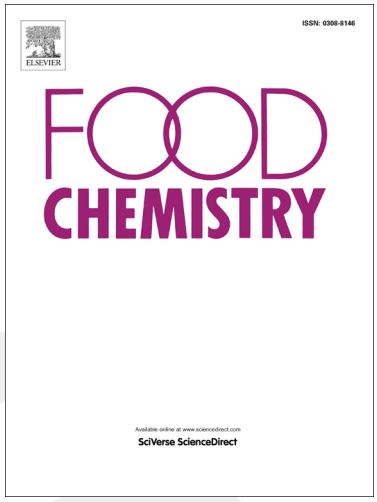

Resistant Starch Type V Formation In Brown Lentil (*Lens Culinaris* Medikus) Starch With Different Lipids/Fatty Acids

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RESISTANT STARCH TYPE V FORMATION IN BROWN LENTIL (*Lens culinaris* Medikus) STARCH WITH DIFFERENT LIPIDS/FATTY ACIDS

Running title: RS5 FORMATION IN BROWN LENTIL STARCH

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Abstract

This study aimed to characterize the brown lentil (*Lens culinaris* Medikus) starch and investigate the formation of amylose-lipid complexes (Resistant Starch Type V) by the addition of different lipids/fatty acids (10%, w/w) to both raw and cooked starch samples. Resistant starch content (measured by the official method of AACCI (Method 32-40), using the resistant starch assay kit) of raw brown lentil starch (BLS) increased significantly by the additions of lipids/fatty acids, starch sample complexed with HSO (hydrogenated sunflower oil) (14.1±0.4%) being the highest. For the cooked starch/lipid complexes, more profound effect was evident (22.2-67.7%). Peak, breakdown and trough viscosity values of the amylose-lipid complexed starches were significantly lower than that of BLS ($p<0.05$), while significant decreases in the setback and final viscosities were only detected in oil samples, but not in fatty acids. Each lipid in concern exerted different effects on the digestibility of starch

and amylose-lipid complex formation while having no substantial differential effects on the thermal properties of starch depicted by differential scanning calorimetry (DSC). Amylose-lipid complex formation with suitable fatty acids/lipids seems a promising way of increasing resistant starch content of food formulations. Although the applications being quite uncommon yet, brown lentil seems to have potential both as a starch and also as a resistant starch source.

Keywords: Brown lentil, resistant starch type-5, amylose-lipid complex, physicochemical properties

1. Introduction

Lentils are usually known with their two main types based on the difference between their seed coat color (green or red). Green lentil (Macrosperma) has green-brown seed coat with a yellow cotyledon while red lentil (Microsperma) has a pale grey- dark seed coat with a red cotyledon. Brown lentil is a member of *Lens culinaris* Medikus and mainly grown around Malatya region in Turkey. It is smaller and more circular in comparison to green lentil. This unique type of lentil is traditionally used in recipes of meatballs, rice and soups.

Starch may be considered as one of the significant components of lentils. Starches may be classified into different groups according to the rates of digestion; rapidly digestible starch (RDS); slowly digestible starch (SDS) and resistant starch (RS). RDS is digested in the small intestine and forms a high glycemic response while SDS is digested at a slower rate, having a relatively lower glycemic response in blood. RS, on the other hand, is not digested in the small intestine, instead fermented in the large intestine by the microorganisms into short chain fatty acids (Englyst, Kingman, & Cummings, 1992). Factors such as granule structure, amylose-amylopectin ratio, starch source etc. were reported to affect the starch resistance to digestion (Finocchiaro, Birkett, & Okoniewska, 2009). Another perspective on digestion kinetics defines two distinct limiting factors such as; 1) barriers that prevent the binding of

enzyme to starch and 2) structural features that slow down the amylase action (Zhang, Dhital, & Gidley, 2015).

The four types of RS in foods are classified as: (1) RS1 – physically inaccessible starch, which is entrapped within whole or partly milled grains or seeds; (2) RS2 – some types of raw starch granules (such as banana and potato) and high-amylose (high-amylose corn) starches; (3) RS3 – retrograded starch (either processed from unmodified starch or resulting from food processing applications) and (4) RS4 – starches that are chemically modified to obtain resistance to enzymatic digestion (such as some starch ethers, starch esters, and cross-linked starches) (Ratnayake & Jackson, 2008). In addition to four main types, a new type, RS5, was also introduced recently (5) RS5 – complexes between amylose and lipids that is resistant to enzymatic digestion (Hasjim, Lee, Hendrich, Setiawan, Ai, & Jane, 2010). Lipids (small non-polar molecules, hydrophobic domains of amphilic molecules such as fatty acids, monoglycerides, and surfactants) form complexes between the hydrocarbon portions of the lipids found in the helical cavity of amylose. This mechanism is dominated by unbranched (linear molecule with α-1,4 linked d-glucose units) glucan chains (Tang & Copeland, 2007; Thomas & Atwell, 1999). RS5 was reported to reduce postprandial glycemic responses, and is reported to have potential for intervening metabolic syndromes such as type-2 diabetes, obesity, hypertension and heart disease (Hasjim et al., 2010).

From the point of crystallinity, amylose-lipid complexes can be evaluated in two different forms: amorphous complex and crystalline complex. Amorphous complex is the one that is produced at lower temperatures such as 25-60˚C, whereas crystalline complex is formed at higher temperatures of 90-100˚C. Crystalline complex is known to be more resistant to amylolytic enzyme hydrolysis than the amorphous one (Hasjim, Ai, & Jane et al., 2013).

Formation of amylose-lipid complexes was previously presented by several researchers. It was revealed that ingestion of bread with palmitic acid-complexed RS5 resulted in

substantially less postprandial plasma-glucose and insulin responses in human subjects in comparison to ingestion of control bread made with wheat flour (Hasjim et al., 2010).

Fatty acids having longer hydrocarbon chains were reported to form enzymatically more resistant amylose-lipid complexes (Hasjim et al., 2010). Enzyme susceptibility of amylose-lipid complex was proposed in two different mechanisms. According to the first mechanism, this complex decreases the extent of granule swelling and therefore the enzyme has difficulties to reach inside of the starch granule. On the other hand, second mechanism states that the amylose-lipid complex is more resistant to digestion by enzymes in comparison to free amylose (Zhang, Huang, Luo, & Fu, 2012).

It was reported that high-amylose starch that was preheated, debranched with isoamylase and complexed with palmitic acid formed an amylose-lipid complex which was resistant to enzymatic digestion (Hasjim et al., 2010). Also some other studies concentrated on addition of structurally different types of lipids (including triglycerides, saturated and unsaturated free fatty acids and phospholipids) to normal corn starch, tapioca starch, waxy corn starch and high amylose corn starch to investigate their effects on enzymatic hydrolysis and physicochemical properties of starch (Ai, Hasjim, & Jane, 2013). The importance of fatty acid type and content on the degree of amylose-lipid complex in wheat starch (Tang & Copeland, 2007; Tufvesson, Wahlgren, & Eliasson, 2003) and differences between waxy and normal wheat starch complexes with three saturated fatty acids (Wang, Wang, Yu & Wang, 2016) and structural properties in model systems comprising fatty acid, protein and starch (Wang, Zheng, Yu, Wang & Copeland) were previously studied. In addition, it was investigated the differences related to different fatty acid types used for lipid-potato starch complex (Kawai, Takato, Sasaki, & Kajiwara, 2012).

Effect of rice flour and different fatty acid sources (palmitic, myristic and stearic acid) on the formation of amylose-lipid complex was also studied previously (Kaur & Singh, 2000).

According to their findings myristic acid had the highest ability to form the complex while stearic acid having the lowest. It was reported that increase in the fatty acid level used and cooking, also increased the extent of amylose-lipid complex (Kaur & Singh, 2000).

In the literature, lentil starch related studies focused on its physiochemical and functional properties (Joshi, Aldred, McKnight, Panozzo, Kasapis, Adhikari, & Adhikari, 2013; Almeida Costa, Queiroz-Monici, Machado Reis, & Costa de Oliveira, 2006; Hoover & Ratnayake, 2002), starch digestibility mechanisms (Kaur, Sandhu, & Lim, 2010; Chung, Liu, Hoover, Warkentin, & Vandenberg, 2008) or starch modification using different techniques such as extrusion or microwave irradiation(Gonzalez & Perez, 2002).

In this study; characterization of BLS as a new source of starch and investigating its raw and cooked combinations with different lipid/fatty acid sources to form amylose-lipid complexes was aimed to provide new data for studies focusing on RS5 formation.

2. Materials and Methods

2.1. Materials

2.1.1. Lentils

Brown lentil (*Lens culinaris* Medikus) samples were supplied from Duru Bulgur Ltd. (Turkey) and stored at room temperature until analysis.

2.1.2. Oil and fatty acids

Corn oil (Kırlangıç, Istanbul, Turkey), extra virgin olive oil (Yudum, Istanbul, Turkey), soy oil (KRK, Istanbul, Turkey) were obtained from local markets, hydrogenated sunflower oil (HSO) was obtained from Unipro Gida (Istanbul, Turkey), palmitic acid (C16:0) and stearic acid (C18:0) were supplied from Sigma Aldrich Company (Germany). Oil samples were stored at room conditions, while fatty acids were stored in the refrigerator at 4°C until analysis.

2.1.3. Chemicals

Ethanol, hexane, maleic acid and sodium hydroxide (Sigma Aldrich) were the main chemicals used during analysis.

2.2. Methods

2.2.1. Starch isolation

Ground brown lentils (100g) were ground using a commercial blender (Waring Commercial, Torrington, CT, USA) to obtain brown lentil flour (BLF). According to the modified method of Gihasi, Hoseney & Varriano-Marston (1982), part of BLF (100g) was mixed with 200 ml of distilled water for 2 min using the blender and sieved through 200-mesh polypropylene sieve. The remaining residue was washed with 200 ml water and the slurry was centrifuged at 1500x*g* for 15 min. Supernatant was discarded while the remaining solid phase is blended again with 200 ml distilled water using a blender and re-centrifuged. The procedure was repeated twice. Tailing starch was carefully separated from the bottom prime starch and was further purified for three times by mixing, blending and centrifuging. All purified starch fractions that were combined and air dried at 20°C, were ground and sieved through a 120-mesh sieve. This final starch obtained was named as brown lentil starch (BLS).

2.2.2. Preparation of amylose-lipid complexes with raw and cooked starches

Isolated BLS (4 g) was mixed with each of the lipids/fatty acids at a ratio of (10%, w/w) in dry weight basis at room conditions.

For cooking treatments; each lipids/fatty acids (10%, w/w) were added one by one into BLS (4 g) and cooked with deionized water (3x, w/w) for 8 minutes in a boiling water bath (□95˚C) with constant manual stirring to have completely gelatinized samples. Samples were dried in an air oven at 45°C and ground to form fine powders with a grinder (IKA®-Werke GmbH & Co. KG, Staufen, Germany) (Ai et al., 2013).

2.2.3. Composition and characterization of brown lentil starch and flour

Both BLS and BLF were analyzed for their moisture (AOAC International Method No. 925.10), ash (AOAC International Method No. 923.03), protein (Kjeldahl Method, AOAC International Method 920.87) and oil contents (Soxhelet extraction). Amylose/amylopectin ratio (K-AMYL, Megazyme Co., Wicklow, Ireland) was also determined in isolated BLS samples.

2.2.4. Starch digestibility

Resistant and non-resistant starch contents were measured by the total resistant starch assay kit (K-RSTAR, Megazyme, Ireland). Measured contents were summed up to give the total starch contents of the samples.

Starch digestibility determinations were made using the modified method of Englyst, Veenstra, & Hudson (1996).

2.2.5. Thermal properties

Thermal properties (transition onset temperature, T_o; transition peak temperature, T_p; and transition enthalpy, ΔH) of both amylose-lipid complexes formed and BLS were investigated using a DSC (Q10; TA Instruments Inc., New Castle, DE, USA). Universal Analysis 2000 Version 4.5A (TA Instruments Inc.) software was utilized. The calibration was made using an indium standard.

2.2.5.1.Thermal properties of brown lentil starch and effect of lipid addition

Samples (5 mg) were weighed into aluminum pans (TA Instruments Inc.) and each pan was hermetically sealed after addition of 15 µl distilled water using a micro-syringe. Sample pans were firstly cooled to 10°C and then heated to 180°C at a rate of 10°C/min. A sealed empty pan was used as a reference. Thermograms were used to calculate thermal parameters (transition onset temperature, T_o; transition peak temperature, T_p; and transition enthalpy,

ΔH). Same procedure was repeated for starch samples mixed with 10% lipid/fatty acid and triplicate measurements were made.

2.2.5.2.Effect of cooking on thermal properties of lipid added samples

Similar procedure was followed to determine the thermal properties of cooked starch-lipid samples. Samples (5 mg) were weighed into aluminum pans (TA Instruments Inc.) and each pan was hermetically sealed after addition of 15 µl distilled water using a micro-syringe. Sample pans were first heated from 10°C to 180°C at a rate of 10 °C/min. Then a cooling procedure with a rate of 40°C/min was applied from 180°C to 10°C. A final re-heating procedure was made from 10°C to 180°C at a rate of 10 °C/min. Thermograms were used to calculate thermal parameters and all measurements were done in triplicate.

2.2.6. Pasting properties

Pasting properties of the brown lentil starch and amylose-lipid complexes were tested using Rapid Visco-Analyzer (RVA 4, Perten Inst.**,** Australia). In this assay, 2.58 g sample (db) were mixed with distilled water to make a total mixture weight of 28.0 grams (containing approximately 8% starch, w/w). Sample was equilibrated at 50°C for approximately 1 min, heated to 95 °C at a rate of 6°C/min, held at 95 °C for 5 min, cooled down to 50 °C at the same rate of 6°C/min. Pasting data was acquired using Thermocline Software (Perten Inst., Australia). Pasting tests were repeated twice for each sample.

2.2.7. Scanning Electron Microscope (SEM) images of samples

Starch powders and their respective lipid complexed powders were fixed on aluminum pin-type stubs using carbon paste. Excess powder was removed using high-pressure air. Samples were coated with gold-palladium by a mini sputter coater (Quorum SC7620; Quorum Technologies Ltd, Laughton, UK). The samples were viewed at 1,500× resolution with a scanning electron microscope (FEI Quanta 250 Company, Hillsboro, OR, USA).

3. Results

3.1. Composition and characterization tests

Total moisture, ash, protein and fat contents of isolated brown lentil starch (BLS) were found as 14.5%, 0.3%, 0.8% and 0.4%, respectively. Brown lentil flour (BLF) measurements, on the other hand were measured as 12.9%, 2.9%, 24.9 % and 2.5%. for moisture, ash, protein and fat contents, respectively. Moisture contents were slightly higher than previous findings in literature (Chung et al., 2008; Kaur et al., 2010). Isolation of starch from lentil flour caused significant ($p<0.05$) decreases in ash, protein and fat contents as expected. In literature, protein contents in red and green lentil samples were found around 28.7-31.5% (Chung et al., 2008) and 24.4-25.8% (Ma, Boye, Simpson, Prasher, Monpetit, & Malcolmson, 2011). These findings were consistent with the present findings for protein content (24.9%) of lentil flour. Fat and ash values were also close to previous findings (Chung et al. 2008; Kaur et al., 2010). Amylose content of BLS was measured as 28.6%. Previous studies on lentil starch measured the total amylose content as 32.52 % and 30.6-31.2% (Joshi et al., 2013; Chung et al., 2008). In present research, amylose content of BLS was found to be slightly lower than the other results found in the literature, which may be originated from the varietal difference.

3.2. Starch digestibility

Resistant starch (RS), rapidly digestible starch (RDS) and slowly digestible starch (SDS) contents of single BLS and combinations of cooked/uncooked BLS samples with one of different lipid sources (at a ratio of 10% in dry weight basis) were depicted in Table 1.

According to a study on *in-vitro* digestibility of starches from different Indian lentil (*Lens culinaris*) cultivars, RDS, SDS and RS contents of *L. culinaris* starches ranged from 56.0 to 65.5%, 5.1% to 9.2%, and 29.4% to 34.8%, respectively (Chung et al., 2008). These researchers suggested that the digestibility of starches may differ among species which was attributed to their variable protein contents (Chung et al., 2008), granule size (Lindeboom,

Chang, & Tyler, 2004), amylose/amylopectin ratio (Hoover & Sosulski, 1991), retrogradation of amylose and/or degree of crystallinity (Chung et al., 2009).

RDS level in BLS (27.4%) was the highest and exhibited a decline after all lipid additions. However, a sharp decline was significant ($p<0.05$) after addition of hydrogenated sunflower oil (HSO) and fatty acids such as stearic and palmitic acids (decrease about 46%) in uncooked samples. Only for olive oil the extent of decrease was around 4.8% for the uncooked sample and 5.5 % for the cooked sample (Depicted also on Table 1) which were not found statistically significant ($p>0.05$). Effect of fatty acid additions on RDS contents was in agreement with previous studies conducted on different starch sources such as potatoes or debranched high-amylose maize starch (Kawai et al., 2012; Zhang et al., 2012), as well. It is mainly about the decrease in the amount of amorphous amylose after destruction of starch granules as a result of gelatinization. Physically and chemically unstable structure of amorphous amylose makes it more prone to hydrolysis while its presence in the complex slows down the rate of hydrolysis (Kawai et al., 2012).

Therefore, the main change in the rate of starch hydrolysis is about the complex formation between amylose and fatty acids (Ai et al., 2013).

Resistant starch content of uncooked BLS sample (9.9%) increased significantly with lipid/ fatty acid additions ($p<0.05$), increase being insignificant ($p>0,05$) for olive oil. Similar increases in RS were also detected in previous studies (Zhang et al., 2012).

Cooking with lipid sources significantly increased the RS contents when compared to BLS including the olive oil. Therefore, the RS content in BLS was measured as 9.9%, and increased to 12.1% in olive oil added and cooked sample. Contradictory results were detected in previous studies. Some researchers reported significant decreases after cooking of lentils and beans (Almeida et al., 2006; Kutos, Golob, Kac, & Plestenjak, 2003) while some other researchers (Osorio□Díaz, Bello□Pérez, Sáyago□Ayerdi, Benítez□Reyes, Tovar, &

Paredes López, 2003; Tharanathan & Mahadevamma 2003) found increases in RS contents similar to present findings of this study. These increases were mainly attributed to amylose retrogradation.

Various effects of cooking were evident on SDS content. Although HSO, stearic acid and palmitic acid additions applied with cooking significantly increased the SDS contents in BLS; soy oil and olive oil additions resulted in decreases ($p<0.05$). In a previous study, SDS contents were reported to decrease after cooking with different lipid sources, however they also reported that starch type was a determinant factor for starch hydrolysis (Ai et al., 2013). The decline in enzymatic hydrolysis was mainly attributed to amylose-lipid complex in previous studies. This complex was related with the restrictedly swollen starch granules that reduced the starch hydrolysis (Ai et al., 2013; Hasjim et al., 2010; Tester & Morrison, 1990).

The effects of incorporating fatty acids (stearic acid, lauric acid, linoleic acid, myristic acid, oleic acid and palmitic acid) into potato starch was studied, previously by Kawai et al. (2012). Their study aimed to investigate the differences related to different fatty acid types used and reported that the extent of amylose-fatty acid complex might be related with the saturation. They suggested that complex was stronger as the number of saturated fatty acids increased (Kawai et al., 2012; Thachil, Chouksey, & Gudipati, 2014). Preference for inclusion of saturated fatty acids into starch granules to form complexes was reported to be higher than of unsaturated fatty acids (Zhou, Robards, Helliwell, & Blanchard, 2007). Degree of complex formation between lipid sources and amylose was related with the preparation and/or pre-treatments such as agitation applied on the complex (Tang & Copeland, 2007). Differences related to amylose; such as starch source amylose chain length and amylose content were also suggested as possible causes of changes in the characteristics of the complexes formed (Kawai et al., 2012).

3.3. Thermal properties by DSC

Thermal properties of BLS, 10% lipid added uncooked and 10% lipid added cooked samples were depicted in Tables 2a) and 2b), respectively. There are two types of amylose-lipid complex was reported based on the crystallinity properties. The first one is produced at lower temperatures (25-60˚C), whereas the crystalline form is at a higher temperature (90-100˚C) (Tufvesson et al., 2003). Isolated BLS was found to have an onset temperature (T_o) of 60.2°C and peak temperature (T_p) of 67.2°C for gelatinization transition. Enthalpy (ΔH) was measured as 10.5 J/s. According to some previous findings, for starch gelatinization of lentil; the onset temperature was measured as 63.1°C, peak temperature as 69.9°C, and enthalpy as 13.5 J/g (Chung et al., 2009). These findings were only slightly higher than present values for BLS. Gelatinization temperatures and enthalpy detected in present study were also consistent with the other values reported in literature (Kaur et al., 2010; Moshi et al., 2013).

Previous studies have suggested the lipid content as a factor for higher transition temperatures, and possible reason of this fact is explained by the enthalpic requirement of lipid as an additional substance in the structure (Dupuis, Liu, & Yada, 2014). Although lipid additions shifted the measured T_o values to slightly higher temperatures; only for stearic acid added BLS the gelatinization transition shifted (68.1˚C) significantly ($p<0.05$). According to Tester (1997), the extent of lipid complexed with amylose chain is among the factors that affect the gelatinization properties; together with other factors such as the molecular structure of amylopectin and amylose/amylopectin ratio. Moreover, Joshi et al. (2013) highlighted that higher transition temperature for corn starch may be attributed to its higher lipid content and more compact granular structure. Moreover, previous research that focused on fatty acids during the formation of amylose-lipid combinations (Tufvesson et al., 2003) revealed that the chain length was a very effective factor for the onset of transition temperature for the fatty acid containing combinations. For T_p values, no substantial change was determined among samples after lipid additions. Slight changes after additions of lipids into starch was similar to

the results found by other researchers who also detected no significant changes in gelatinization properties after formation of amylose-lipid mixtures (Ai et al., 2013).

Overall, RS5 is more heat stable than most RS2 starches, as shown by having a higher transition onset temperature than native starch (Fuentes□Zaragoza, Sánchez□Zapata, Sendra, Sayas, Navarro, Fernández□López, & Pérez□Alvarez, 2011). It is due to the fact that a lipid component is present, which also has its own enthalpic requirement for heating. On heating, the complex may dissociate, but upon cooling it will spontaneously revert back to its complexed form (Hasjim et al., 2013).

It was reported that, when analyzed in DSC in the presence of excess water amylose-lipid complex of granular starch showed a dissociation temperature in the range of 70-108^{o}C (Hasjim et al., 2013). In previous researches endothermic peaks of amylose-lipid complexes were reported to form in the range of 100-112^{o}C for barley (Szczodrak & Pomeranz, 1992) and at around 101.3^{o}C for corn starch (Ai et al., 2013). No amylose-lipid dissociation peak was present for native BLS. Low amount of endogenous lipids and relatively low amount of amylose present in BLS was proposed as the reason for the absence of the peak (Ai et al., 2013). Therefore, addition of lipid sources resulted in the formation of amylose-lipid complex dissociation peaks.

Gelatinization has an important role on RS5 formation. Amorphous regions of starch granules tend to destabilize when starch is heated in the presence of water. This phenomenon generally takes place through water absorption and swelling. Moreover, the crystalline regions (which consist mainly of double helices of amylopectin) become unfolded and this whole process is generally accepted as starch gelatinization (Kawai et al., 2012). The starch granules are destroyed as gelatinization progresses and amylose leaks from the granules. The leaked amylose granule forms inclusion complexes in the presence of fatty acid or surfactant such as a monoglyceride, between the hydrocarbon portion of the lipid located within the helical

cavity of amylose (Tang & Copeland, 2007, Thomas & Atwell, 1999). Structure of amylose-guest helixes is generally organized in crystals of V amylose types and formation of this complex not only retards the retrogradation progress, but also has been suggested to affect the starch digestibility by the decrease in water solubility (Tang & Copeland, 2007).

For BLS and 10% lipid added uncooked samples (Table 2a), HSO and stearic acid added BLS samples had the highest T_o values of 106.4°C and 106.1°C, respectively for amylose-lipid complex dissociation. Palmitic acid, corn oil, soy oil and olive oil additions resulted in significantly lower ($p<0.05$) T_o values (102.6, 99.2, 101.5 and 98.7°C, respectively). The T_p values were also measured for stearic acid (109.8°C), HSO (109.6°C), corn oil (107.7°C) and palmitic acid (107.1°C) added samples, respectively. T_p measured for soy oil and olive oil added samples were significantly lower ($p<0.05$) than the rest of the samples.

The temperature for amylose-lipid complex dissociation peak has been suggested to shift as the length of the hydrocarbon chains of lipids increased, while to shift further with the increase in the number of double bonds (Tufvesson et al., 2003). Long hydrocarbon chains were associated with the stronger interactions with the hydrophobic cavity of the amylose helix, whereas palmitic acid with a straight but shorter hydrocarbon chain had weaker interaction (Ai et al., 2013). Previous results on results incorporating fatty acids also revealed that higher T_p values might be associated with a longer helical length and thus a more stable complex between starch and lipid (Kawai et al., 2012). Saturation is claimed as another factor to shift the amylose-lipid complex dissociation (Tufvesson et al., 2003), therefore HSO had the relatively higher peak onset and peak temperatures, as well. HSO had also the lowest enthalpy among all samples ($p<0.05$).

Gelatinization peaks were lost in 10% lipid added cooked samples, therefore that means they were completely gelatinized. However, amylose-lipid complex dissociation peaks were detected in the same samples. The peaks of amylose-lipid complex dissociations in cooked

samples were generally larger, when their enthalpy values were compared with the uncooked samples. These findings for the effect of cooking were parallel to the findings in previous studies and was attributed to the stronger effect of cooking than only physical mixing on the amylose-lipid complex formation (Ai et al., 2013). Rescanning was applied to confirm the presence of amylose-lipid complex (Hasjim et al., 2010). After rescanning, decrease and/or complete loss of enthalpy values was evident. Generally, T_p values were also detected to decrease in all samples in comparison to amylose-lipid complex dissociations (Details were depicted on Table 2b).

3.4.Pasting properties

The effect of amylose-lipid complex formation on RVA pasting properties of BLS were shown in Table 3. Peak, breakdown and trough viscosity values of the amylose-lipid complexed starches were significantly lower than that of brown lentil starch ($p<0.0.5$). The main reason of the low viscosity values is the restriction of the granule swelling with the addition of lipids/fatty acids, as stated by some of the other researchers (Tester & Morrison, 1990; Debet & Gidley; 2006, Ai et al., 2013). Addition of fatty acids significantly decreased the peak viscosity, while increasing the setback and final viscosity values. Similar findings were supposed to be related with the melted and then re-solidified structure of fatty acids during RVA heating-cooling cycle, which elevated the set-back and final viscosity measurements (Ai et al. 2013).

3.5. SEM Images

When SEM images are evaluated; BLS was found to have oval granular shape (Figure 1a) and brown lentil flour had a more complex structure with protein molecules bound to starch granules (Figure 1b). SEM images of BLS displayed characteristic shapes of lentil starch granules, in smooth sphere and elliptical shapes, that has also been defined as round, oval and

irregular (Ahmed, Taher, Mulla, Al-Hazza, & Luciano, 2016) and without any fissures (Kaur et al., 2010), previously.

Diameters of the BLS granules ranged between 10-20 µm, according to the present study. This finding was similar to previous findings in the literature which reported the characteristic particle diameter of the *L. culinaris* starches in the range of 15.9 and 17.4 µm), average 22.5 µm (Ahmed et al., 2016) and 16–19 µm (Yoshimi & Toshiko, 2006). Previous studies suggested that starch granule size may be effective on the physicochemical properties, such as gelatinization and pasting, enzyme susceptibility, crystallinity and solubility (Lindeboom et al., 2004).

For lipid added raw starch samples transparent granular structures were evident (Figure 2). In contrast, when the images of cooked starch samples are evaluated (Figure 3) it was seen that granular structures of starch were collapsed and crystalline structures became more evident because of the cooking and cooling process.

Cooked samples possess totally a different structure (Figure 3). Destruction of the native, crystalline granular structure during cooking which is generally detected is considered as an expected result for starch sources (Moore, Ai, Chang, & Jane, 2015). A complete destruction of structure was detected in stearic acid and olive oil added and cooked samples. In contrast, a moderately protected granular structure in corn oil and soy oil added BLS was evident. In contrast, HSO and palmitic acid additions gave a completely different solid, crystalline structures.

According to previous findings; the organized structure of amylose-lipid complex were reported to become less ordered related with the degree of saturated and unsaturated fatty acids (Zabar, Lesmes, Katz, Shimoni, & Bianco-Peled., 2009). Moreover, particle size of the complex formed was higher and broader, in relation with the unsaturation (Lesmes, Cohen, Shener, & Shimoni, 2009).

4. Conclusions

The results of this study presented detailed data about some of the physicochemical properties of BLS and depicted its potential as a starch source. Moreover, its complexes with lipid sources, especially with saturated lipids, has displayed positive improvements in terms of starch digestibility. Therefore, BLS complexes with lipids seem promising for applications in food products. Particularly, those complexes may be used as starch alternatives in the food formulations especially for consumers requiring lower insulin responses.

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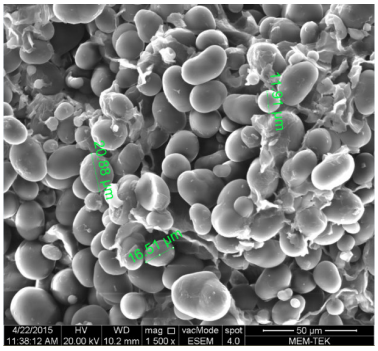
Figure Captions

Figure 1. Scanning electron microscope (SEM) micrographs of (A) BLS and (B) Brown lentil flour. Magnification bars in the SEM micrographs represents 50 μm

Figure 2. SEM micrographs of BLS (uncooked) with added lipids. (A): Soy oil added; (B): Olive oil added; (C): HSO added; (D): Corn oil added; (E): Stearic acid added; (F): Palmitic acid added. Magnification bars in the SEM micrographs represents 50 μm

Figure 3. SEM micrographs of cooked BLS with added lipids. (A): Soy oil added; (B): Olive oil added; (C): HSO added; (D): Corn oil added; (E): Stearic acid added; (F): Palmitic acid added. Magnification bars in the SEM micrographs represents 100 μm.



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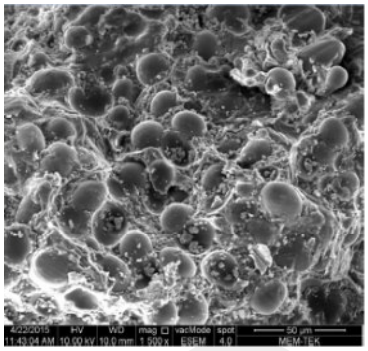

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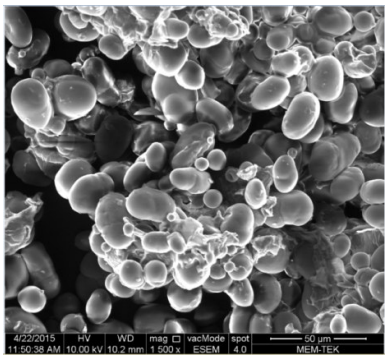

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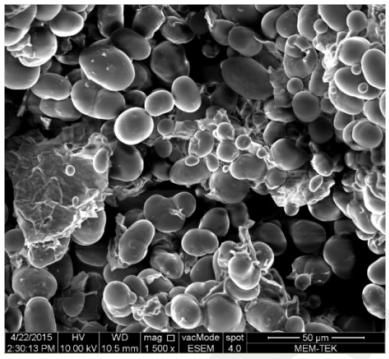

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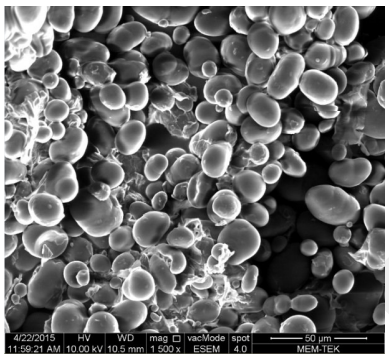

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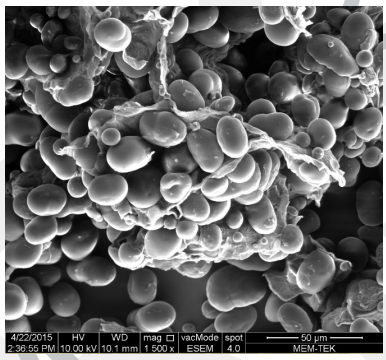

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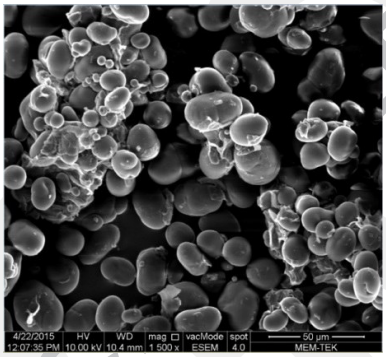

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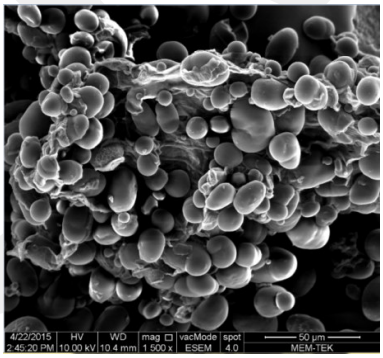

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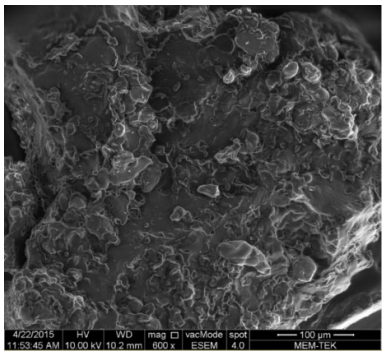
Figure 2.



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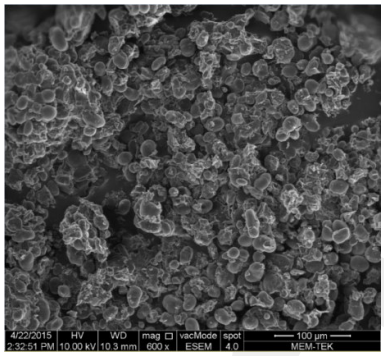

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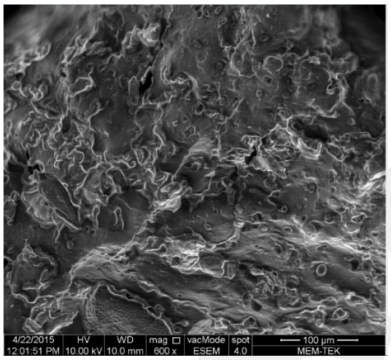

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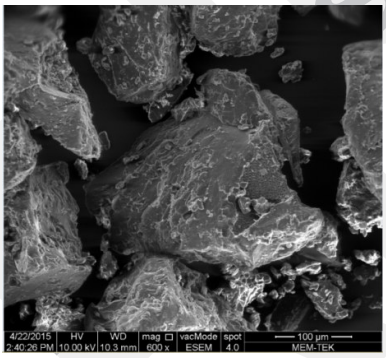

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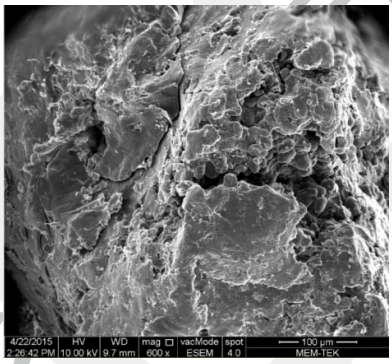

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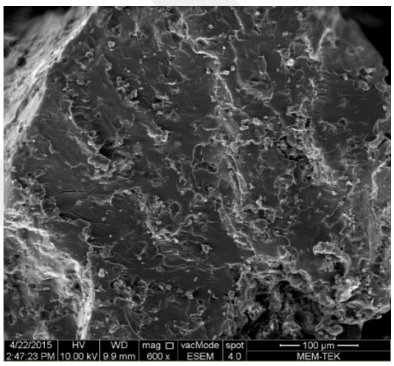

(F)

Figure 3.

Table 1. Resistant starch (RS), rapidly digestible starch (RDS) and slowly digestible starch (SDS) contents of single BLS and combinations of cooked/uncooked BLS samples with one of different lipid sources (at 10% ratios in dry weight basis)*

Sample		RS %	RDS %	SDS%
Control- native starch	BLS (Amylose content 28.6%)	9.9±1.03j	27.4±1.05a	62.5±1.84c
Uncooked	BLS + corn oil	11.8±0.23hi	24.6±0.72cd	62.9±0.93c
		+19.2	***-11.2***	***+0.6***
	BLS + soy oil	13.0±0.85fg	23.9±0.41de	62.9±1.07c
		+31.3	***-12.8***	***+0.6***
	BLS + olive oil	10.7±0.09ij	26.1±0.56ab	62.9±0.93c
		+8.1	***-4.8***	***+0.6***
	BLS + hydrogenated sunflower oil	14.1±0.44def	14.8±0.36f	71.2±0.49a
		+42.4	***-46.0***	***+13.9***
	BLS + stearic acid	13.5±0.08ef	14.6±0.35f	71.7±0.61a
		+36.4	***-46.7***	***+14.7***
	BLS + palmitic acid	14.9±0.64cd	13.8±0.31fg	70.9±0.52a
		+31.3	***-14.6***	***+13.5***
Cooked	BLS + corn oil	13.0±0.04fg	23.4±0.43de	63.5±1.04c
		+31.3	***-14.6***	***+1.6***
	BLS + soy oil	15.2±0.46bc	22.9±0.64e	61.9±0.33c
		+53.5	***-16.4***	***-1***
	BLS + olive oil	12.1±0.08gh	25.9±0.68bc	59.0±0.83d
		+22.2	***-5.5***	***-5.6***
	BLS + hydrogenated sunflower oil	16.6±0.06a	14.5±0.40f	68.8±0.78b
		+67.7	***-47.1***	***+10.1***
	BLS + stearic acid	14.4±0.06cde	12.2±0.28h	71.5±0.42a
		+45.4	***-55.5***	***+14.4***
	BLS + palmitic acid	16.3±0.08ab	12.7±0.28gh	70.9±0.41ab
		+64.6	***-53.7***	***+13.4***

*The mean value ± standard deviation of duplicate analyses are given. Values with different letters within the same column differ significantly ($p<0.05$). Lines in bold italics represent the %change in RS, RDS and SDS% contents of amylose-lipid combinations, when compared to values of BLS.

Table 2a). Thermal properties of BLS and 10% lipid added uncooked samples*

	Starch Gelatinization			Amylose-lipid complex dissociation		
Sample	**T_o (°C)**	**T_p (°C)**	**ΔH (J/g)**	**T_o (°C)**	**T_p (°C)**	**ΔH (J/g)**
BLS	60.2±0.6 b	67.2±2.66 abc	10.5±1.97 ab	ND	ND	ND
BLS + corn oil	63.8±1.0 b	72.1±1.75 a	13.9±2.72 a	99.2±1.24 c	107.7±1.64 ab	2.4±1.35 b
BLS + soy oil	61.2±0.6 b	66.3±2.13 bc	14.1±2.94 a	101.5±0.89 c	103.7±2.28 bc	0.6±0.46 b
BLS + olive oil	63.7±1.5 b	67.6±0.49 ab	14.8±2.63 a	98.7±1.95 c	100.6±2.76 c	1.5±0.46 b
BLS + HSO	63.2±2.8 b	65.2±2.29 bc	14.9±2.06 a	106.4±2.14 ab	109.6±3.18 a	7.1±1.54 a
BLS + stearic acid	68.1±0.7 a	70.1±1.64 ab	7.3±1.59 b	106.1±2.53 a	109.8±2.12 a	0.5±0.12 b
BLS + palmitic acid	60.5±0.2 b	62.2±0.39 c	9.7±1.07 ab	102.6±0.47 bc	107.1±0.53 a	0.4±0.07 b

2b). Dissociation of amylose-lipid complex of 10% lipid added cooked samples*

	First Scanning (Amylose-lipid complex dissociation)			Rescanning		
Sample	**T_o (°C)**	**T_p (°C)**	**ΔH (J/g)**	**T_o (°C)**	**T_p (°C)**	**ΔH (J/g)**
BLS + corn oil	96.0±2.30 b	99.1±2.07 b	1.0±0.08 b	91.0±1.95 b	92.2±3.12 b	0.1±0.06 c
BLS + soy oil	98.0±2.46 b	100.8±3.26 ab	8.2±2.74 a	92.4±4.51 b	94.8±4.16 b	6.9±2.33 a
BLS + olive oil	93.5±0.32 b	96.9±2.36 b	4.9±0.98 a	89.7±1.77 b	90.4±2.52 b	1.3±0.09ab
BLS + HSO	102.9±1.78 a	105.7±2.18 a	0.17±0.01 b	ND	ND	ND
BLS + stearic acid	95.9±1.35 b	99.1±1.96 b	0.6±0.17 b	97.2±2.03 a	97.9±2.30 a	0.1±0.02 c
BLS + palmitic acid	ND	ND	ND	ND	ND	ND

*Different letters (a–d) within the same column differ significantly ($p < 0.05$). T_o is the onset temperature T_p is the peak temperature ΔH is the enthalpy of transition.
N.D.: not detectable.

Table 3. Pasting properties of the 10% lipid added samples*

Sample	Peak Viscosity (cP)	Breakdown Viscosity (cP)	Trough Viscosity (cP)	Setback Viscosity (cP)	Final Viscosity (cP)
BLS	2314±19.8 a	1117±9.9 a	1197±9.9 a	2775.5±53 b	3972.5±43.1 a
BLS + corn oil	1686±48.1 b	845±70.7 b	841±22.6 cd	2077±70.7 c	2918±48.1 b
BLS + soy oil	1705±170 b	960±120.2 ab	745±49.5 d	2038.5±92.6 c	2783.5±43.1 b
BLS + olive oil	1685±31.1 b	871±25.5 b	814±56.6 cd	1988±0.0 c	2802±56.6 b
BLS + HSO	1660±26.9 b	608±14.1 c	1052±12.73 b	1762.5±55.9 d	2814.5±43.1 b
BLS + stearic acid	1468.5±33.2 bc	414±0.0 c	1054.5±33.2 b	3001±0.0 a	4055.5±33.2 a
BLS + palmitic acid	1366.5±36.1 c	413.5±0.7 c	953±35.4 bc	3001±0.0 a	3954±35.4 a

*Different letters (a–d) within the same column differ significantly ($p < 0.05$).
cP: Centipoise

Highlights

- Brown lentil may be used both as a starch and as a resistant starch source
- Lipid/fatty acid additions substantially increased resistant starch content
- The highest amount of RS5 was detected in the hydrogenated sunflower oil complex
- Cooking increased the RS5 formation in brown lentil