

## Influence of storage time and starches on texture attributes of conventional milk yogurt using response surface methodology

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### Article history

Received: 2 August 2016  
Received in revised form:  
19 August 2016  
Accepted: 21 August 2016

### Abstract

This research compared the effects of two types of starch (corn and kudzu) at different concentrations on the texture sensory attributes of conventional milk yogurt. The effects of days of storage, starch type and concentration, and their interactions on nine sensory variables were determined by the use of covariate analysis. Time and starch interaction were significant for only two sensory responses. All but graininess attribute had a significant time response and seven attributes responded to the concentration of starch. Best-fit polynomial regressions over time were determined for the responses affected. Response surface methodology (RSM) was applied to determine the effects of starch type and concentration on yogurt products. In addition, scanning electron microscopy study revealed a difference in microstructure of starch in yogurt by time. Kudzu starch may be used as an alternative starch in yogurt applications.

### Keywords

Yogurt  
Kudzu starch  
Corn starch  
Textural sensory  
RSM,  
SEM

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### Introduction

Kudzu, the dried root of *Pueraria lobata* (Wild.), is one of the most important edible herbs used in oriental medicine (Hung and Morita, 2007). It is a wild perennial vine with very large tuberous roots. Starch comprises about 20% of the fresh roots. Starch composition of *Pueraria lobata* was 58.96% (% of dry weight basis) (Du *et al.*, 2002). Chemical compositions of kudzu root has been widely investigated and it was found to contain high amounts of isoflavones, puerarin, diadzin, daidzein, genistin, genistein, formononetin and their derivatives (Hung and Morita, 2007; Chen *et al.*, 2012; Kayano *et al.*, 2012). Modern studies of kudzu have shown that its extract exhibits antioxidant activities (Guerra *et al.*, 2000). It has also been used for the treatment of alcohol abuse (Lin *et al.*, 1996), hypertension and cardiovascular diseases (Keung, 2002).

The starch of corn, maize (*Zea mays*) represents 80% of the starch produced worldwide. It is inexpensive starch used widely in the food industry for the development of various textures and viscosity and appropriate for several technical applications in products (Jobling, 2004). Clarity, flavor, and durability to withstand rigorous processing conditions

are among its common properties (Whistler *et al.*, 1984). The corn starch granules are known to have pores or channels on the surface with a polygonal shape (Geng *et al.*, 2007). According to Geng *et al.* (2007), kudzu starch and corn starch have similar amylose contents.

Starch contributes greatly to the textural properties of many foods including yogurt such as thickening and gelling agents to increase its viscosity and to prevent syneresis (Singh *et al.*, 2003; Tamime and Robinson, 2007). Ares *et al.* (2007) studied the effect of the addition of gelatin and starch on the rheological and sensory properties of sweetened plain-stirred yogurt. Their research showed that the addition of gelatin and starch to yogurt significantly affected sensory and textural parameters and had a significant effect on the occurrence rate of syneresis. The effect of fat replacers upon the texture and microstructure of reduced-fat yogurt was studied (Sandoval-Castilla *et al.*, 2004). Modified tapioca starch was exhibited more relaxed, and loosened structure in all yogurts, with some solubilized starch molecules integrated into the casein micelle network. Grygorczyk *et al.* (2013) study revealed that yogurt texture was an important factor affecting consumer liking. Firmness, creaminess, viscosity, mouth-

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feel and syneresis are considered among the most important descriptors for the textural perception of yogurt (Muir and Hunter, 1992; Jaworska *et al.* 2005; Ares *et al.* 2007; Coggins *et al.* 2008).

Kudzu starch is more cohesive in gel form than other starches. It is more elastic, resists crumbling and present gel stability superior to all other natural starches due to its temperature-time viscosity characteristics. It is not only a superior gelling agent, but offers the advantages of paste (gel) stability. As a result of its fine granule size and other features, it is also most absorbent natural starch. Unlike, other natural starches, kudzu starch produce a stable, very clear, colorless, flavorless, soft, transparent gel (Keung, 2002). *Pueraria lobata* has average size 12 micrometer, amylose content 19-24%, gelatinization temperature 60-72°C, and endothermic peak/gelatinization 60-68.5°C.

To our knowledge, kudzu starch has not been studied in yogurt. The purpose of this study were to investigate the influence of kudzu root starch in conventional milk yogurt in comparison to corn starch and to determine the textural properties of yogurt with kudzu root starch and corn starch.

## Materials and Methods

The commercial Japanese kudzu root starch was obtained from Wild Organic Kudzu (Mitoku Co., Asheville, NC, US), and the modified corn starch (stabilizer; Dairyblend TG-AG) was obtained from TIC Gum Company (White Marsh, MD, US).

### *Yogurt milk preparation*

Yogurt milk preparation procedures of Imamoglu *et al.* (2015) was used. The yogurt base was made with skim milk, cream, nonfat dry milk (low-heat Grade A nonfat milk; Danish Creamery Assoc., Fresno, CA, US), and stabilizer (Continental Colloids, Inc., West Chicago, IL). The yogurt milk was formulated by adjusting skim milk to 1% milk-fat using 36% cream. The skim milk and cream were obtained from the Custer Dairy Processing Plant at Mississippi State University. Nonfat dry milk (NDM) (Danish Creamery Assoc., Fresno, CA, US) was added at a rate of 4% along with 1%, 2%, 2.5%, or 3% w/w (Bourgouin, 1993) commercial stabilizer (modified food starch, carrageenan, and pectin; Continental Colloids, Inc., West Chicago, IL, US). The same procedure was repeated using kudzu root starch (Mitoku Co., NC, US) at the same rates. These ingredients were mixed, heated (at 50°C), and homogenized using a HTST system (Model #- AT20 DF, Paul Mueller Company, Springfield, MO, US)

(Tamime and Robinson, 2007). The processing of yogurt milk was repeated to produce three batches.

### *Yogurt production*

The yogurt production procedures of Imamoglu *et al.* (2015) were utilized and the yogurt samples were stored at 5°C from 0 to 49 days.

### *Yogurt treatments experimental design*

Yogurt samples were produced for the two starch types at four concentrations in three batches (replications). All formulated samples were immediately refrigerated at 5°C and stored from 0 to 49 days. A control yogurt without corn or kudzu starch was also produced.

### *Storage of yogurt*

The storage of yogurt procedures of Imamoglu *et al.* (2015) was followed. The stirred yogurt samples were scooped into 170 ml polypropylene cups (Landis Plastics, Inc. Chicago Ridge, IL), and the lids were sealed. The samples were immediately stored in well-ventilated incubators at 5°C (BOD30A14, GS LabE9, Revco, Asheville, NC). Prior to sensory evaluation, the yogurt samples were removed from the incubators, placed on plastic trays, and taken to a sensory preparation area within 10 min. The trays had a 340 ml opaque plastic cup (Sysco, Memphis, TN) of distilled water, a 340 ml expectorate cup (Sysco, Memphis, TN), unsalted crackers (Premium Brand, Nabisco), a sensory instrument, white plastic spoons (Sysco, Memphis, TN), and napkins (Kroger Brand, Cincinnati, OH). Trays with the yogurt samples were randomly assigned to the panelists for evaluation.

### *Sensory evaluation of yogurt*

Descriptive terms were generated for this study by the six panelists from individual evaluations of the yogurt. Samples were evaluated for hand texture and mouth feel texture). Descriptors were texture hand-felt (slipperiness, firmness, ropiness, viscosity, cohesiveness and denseness), texture oral (firmness, slipperiness and graininess), mouthfeel attribute (firmness, mouthfeel drying, throat-burn intensity, mouth coating, viscosity). The procedures of Coggins *et al.* (2008; 2010) were utilized in this study.

The yogurt samples stored at 5°C were evaluated on days 1, 7, 14, 21, 28, 35, 42, and 49. For scoring, a 15-point intensity scale (where 0 = none and 15 = extremely intense) was used. For each session, 12 samples (4 different concentrations in 3 replicates or batches) were tested, and three sessions were scheduled per month. The panel was trained in the use of the chosen attributes (Coggins *et al.*, 2008)

for 72 h over a 6-month time period (3 h/week). The yogurt cups, brought to ambient temperature, were served with water (Magnolia Springs, MS) and unsalted crackers (Premium brand; Nabisco Foods; Subsidiary of Kraft Foods, Glenview, IL), which were used as palate cleansers between samples. The testing was performed in a sensory laboratory equipped with individual booths and artificial daylight (fluorescent). Panelists were given freshly made (day 1) plain yogurt samples without any starch prior to sensory evaluation. Samples were run in triplicate with a randomized order of presentation to the panelists. The yogurt samples were identified by a three-digit random number code. Expectoration of each sample was encouraged. Formal descriptive round table forums were completed on the yogurts. Approval for this research was received from IRB Docket # 09-126. The protocol was followed for Human Subject Research (Mississippi State University Internal Review Board procedures specifying work with human subjects).

*Statistical analyses*

Statistical analysis was executed using the procedures of SAS 9.1 (SAS, 2003). Covariance analysis (PROC MIXED) was executed on each sensory attribute to test for a significance of the effects of two starches, at four concentrations, evaluated for 1 to 49 days and their interactions. When the interactions of two variables were significant, data was further analyzed by holding one variable constant while analyzing for the response of a second variable. If attribute values changed with the days of storage and the concentration of starch, then response surfaces were generated to help visualize the simultaneous effects of starch concentration and days of storage on attribute intensity. If only one of the two quantitative variables had a significant effect, then a polynomial regression was fit using SAS procedure (PROC REG). With numerous statistical tests, the significance level for fitted models was set at P= 0.01 to control Type I error.

Polynomials fit to attribute values were linear, quadratic, or cubic. The best model was determined as a function of non-reducing adjusted R-square value and the parsimony principal. Curves, such as a linear time response for 1% kudzu starch and a linear time response for 1% corn starch, were compared where appropriate, in order to see if they were significantly different by using the general linear model's significance of residual sum of squares approach (Ryan, 2007).

Table 1. Regression equations and coefficient of determination describing the effect of days of storage and concentration of starch on texture hand-felt and texture oral attributes

(Regression equation: $a+b\text{Day}+c\text{Day}^2+d\text{Concentration}$ )

Attributes	Starch	Constant number	Day	Day <sup>2</sup>	Concentration
<b>Texture Hand-Felt</b>					
Slipperiness	*	6.6	0.037		-0.354
Firmness	Kudzu	4.74	0.35	-0.007	+1.32
	Corn	6.03	0.34	-0.007	+0.96
Ropiness	*	1.76	-0.016		-0.222
Viscosity	*	11.1	+0.183	-0.0029	+0.55
Cohesiveness	*	9.16	-0.085		
Denseness	Kudzu	4.9	+0.26	-0.0044	+1.35
	Corn	6.6	+0.22	-0.0042	+1.02
<b>Texture Oral</b>					
Firmness	*	2.9	+0.356	-0.007	+1.28
Slipperiness	*	4.74	+0.192	-0.0046	-0.438
Graininess	Kudzu	3.02	+0.156	-0.003	
	Corn	2.59	+0.156		

\* indicates kudzu and corn starches have same response to attributes

All regressions have a highly significant P (≤0.01).

*Microstructure analysis of kudzu root starch and corn starch and their structures in yogurt- SEM*

The shape and size of starch granules were observed by SEM (Ziss EVO 50 VP Scanning Electron Microscope, Peabody, MA, US). Kudzu and corn starches were mounted on an aluminum stub with carbon cement adhesive. The samples were coated with gold/palladium (Au/Pd) and micrographs were taken using a SEM apparatus (Zeiss Evo 50) at an accelerating potential of 15 kV. Yogurt samples were fixed for 8 hours at room temperature in a 3% glutaraldehyde solution in 0.1 M phosphate buffer, pH 7.2. After fixation, specimens were rinsed in several changes of 0.1 M phosphate buffer, 7.2 and left overnight in the similiar buffer. Immediately afterwards, the samples were post-fixed in 2% osmium tetroxide (OsO<sub>4</sub>) (Sigma Chemical Co., MO, US) and 0.1 M phosphate buffer at room temperature for 4 hours and rinsed for 30 minutes each in 3 changes of distilled water. The specimens were dehydrated in an ethanolic solutions with increasing concentrations of ethanol (35%, 50%, 70%, 95% for 15 minutes and 4-6 times at 100% for 15 minutes) and then dried using critical point drying in Polaron E-300 CPD (Quorum Technologies, Newhaven, UK) critical-point dryer with liquid carbon dioxide. Dry sections were mounted on aluminum stubs, and sputter coated with Au/Pd. Specimens were imaged and digital images acquired using SEM.

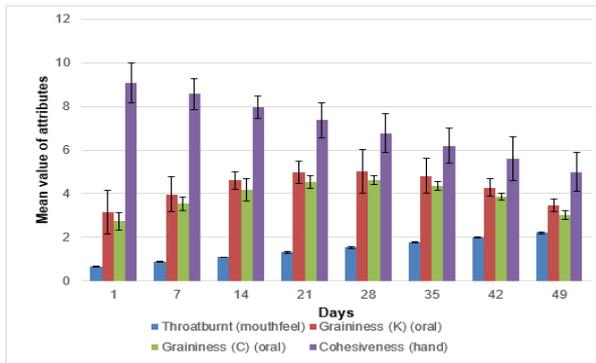


Figure 1. Effect of time on texture attributes of conventional yogurt with starches (kudzu/corn); K: kudzu; C: corn

## Results and Discussion

### Texture sensory analysis

The analysis of variance shows the significance of starch type concentration and storage effects and their interactions. These analyses showed that while none of the texture hand- felt attributes were affected by starch, all hand- felt texture attributes (slipperiness, firmness, ropiness, viscosity, cohesiveness and denseness) were affected by storage duration. Starch concentration affected slipperiness, firmness, ropiness, viscosity, and denseness attributes. There was no significant starch concentration type or starch or day starch concentration interaction. Only denseness texture hand-felt attributes was affected by day x starch interaction. There was a significant quadratic effect for storage days on firmness, viscosity and denseness attributes. A significant quadratic starch concentration by starch effect was found for firmness and denseness.

Textures (hand-felt and oral) of yogurts were significantly affected by starch concentrations and/or storage time. Cohesiveness had the simplest response of the texture attributes. It was unaffected by type of starch or concentration of starch and was linear over day (Table 1). The score value of cohesiveness decreased linearly from 9 to 5 after 49 days (Figure 1). The score of cohesiveness began with 9 on day 1 and decreased to 5 over storage time (day 49). Graininess was affected by the type of starch while it was unaffected by concentration of starch. There was a quadratic effect for storage days on graininess attribute (Table 1). The responses are then two, parallel curves which are quadratic over days of storage (Figure 2). The attributes texture hand-felt firmness and denseness had different responses for each starch (Table 1) and was affected by concentration of starch and by storage days. The response surfaces were similar conic sections with highest attribute value for an intermediate number of storage days and a linear

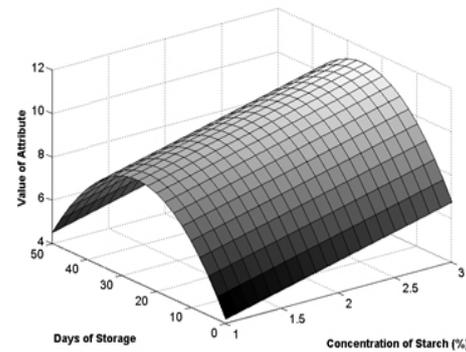
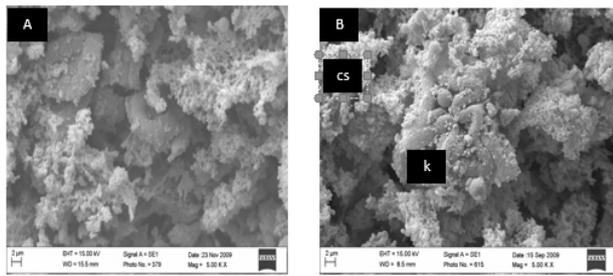


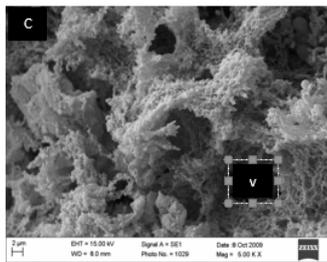
Figure 2. Response surface for firmness of texture oral attribute for yogurt with kudzu/corn starch with respect to day and starch concentration (0-3%)

increase for increasing starch concentration. For these two attributes the corn was consistently one unit greater than kudzu. Two other attributes, texture hand- felt viscosity and texture oral firmness had similar responses with maximum value of attribute at intermediate storage day but an increase in attribute value for increasing concentration of starch (Figure 2). The intensity for firmness attribute peaked at about day 25 for any given starch concentration and the corn starch averaged about 2 points greater intensity than kudzu. Increasing the starch concentration increased the attribute intensity for any given day. Unlike Ares' study (2007), the attribute ropiness was unaffected by type of starch, but had a complex response though attribute values were less than 2. The attribute value of ropiness was maximized at lowest starch concentration on the first day. Increasing the starch concentration reduced the attribute value ropiness and its score was reduced over days. As indicated in hand felt texture, the same is true for texture oral and can be explained by moisture loss. The addition of starch to yogurt results in increased firmness, denseness and viscosity for texture hand-felt and texture oral attributes. Mohammad (2004) found that seven different stabilizers (pectin, guar gum, carboxymethylcellulose (CMC), carrageenan, sodium alginate, corn starch and gelatin), storage time and total solids combined with the thickeners significantly affected the properties such as syneresis and texture of the yogurt samples. Also, starch concentration was an important factor for texture hand-felt attributes except cohesiveness. Only graininess oral texture attribute was affected by starch. Firmness and graininess texture oral attributes were affected by time (day). There was a significant quadratic effect (Day 2) on all texture oral attributes. Williams *et al.* (2003) reported that the addition of starch has beneficial effects on yogurt properties. Graininess encountered in stirred yogurts with added starch can be reduced by choosing appropriate



A: 7 days

B: 28 days



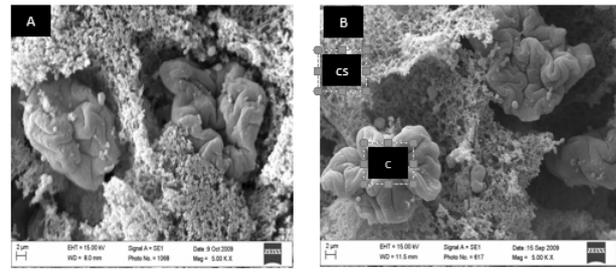
C: 49 days

Figure 3. Scanning electron microscopy (SEM) micrographs of yogurt with 2 % kudzu starch treatment for over storage time: A: 7 days, B: 28 days, C: 49 days. Magnification 5K. Scale bar 2 µm, v, void space; cs, casein; k, kudzu starch.

formulations and fermentation conditions (Williams *et al.*, 2003, 2004).

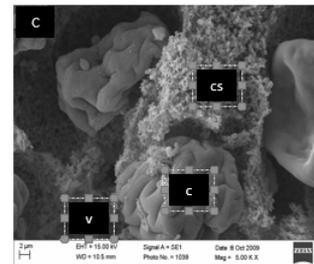
Viscosity had a quadratic response with a maximum rating on day 31.5 with a steady decline thereafter. Viscosity increased in intensity due to loss of moisture and syneresis of the yogurt product until day 31.5 in this study. As indicated in Figure 3 and 4, firmness texture hand-felt and texture oral (Figure 2), the same is true for these texture attributes and can be explained by moisture loss, by the physical changes due to storage and starch in the texture of the yogurt. Predicted value was related linearly to concentration; as concentration increased the attribute score increased. According to study of Singh and Byars (2009), modified starch can form composites with proteins, leading to significant syneresis reduction. Ibrahim and Khalifa (2015) reported that the increase of viscosity in camel's milk yoghurt containing different ratios of modified starch may be due to the interaction between the modified starch and casein particles thus contributing a strong gel when the concentration was increased.

Figure 2 depicts response surface for firmness of texture oral attribute for yogurt with kudzu/corn starch. The response plot was similar to the model of the plot for firmness, viscosity, and denseness of texture hand- felt attribute except attribute value was less. It can be observed the maximum intensity near day 30 and starch concentration maximum, 3. There was a quadratic day effect and linear concentration



A: 7 days

B: 28 days



C: 49 days

Figure 4. Scanning electron microscopy (SEM) micrographs of yogurt with 2 % corn starch treatment for over storage time: A: 7 days B: 28 days, C: 49 days. Magnification 5K. Scale bar 2 µm, v, void space; cs, casein; c, corn starch.

effect on attribute value.

#### Scanning electron microscopy (SEM)

Kudzu starch-yogurt microstructure exhibited protein clusters with some pores and more interconnected clusters of densely integrated protein particles (Figure 3). SEM failed to reveal any notable difference between 7-day and 28-day storage of kudzu-yogurt treatments (Figure 3A and Figure 3B) whereas 49-day yogurt with kudzu starch had more porosity protein network (Figure 3C). Kudzu starch molecules surrounded by casein micelle chains were observed in Figure 3A. The casein micelles from the starch granules can be seen in Figure 3B, and Figure 3C.

In Figure 4A, corn starch granules embedded in micelle protein structure. Also, the starter culture cells, the streptococci and lactobacilli, are observable in Figure 4B. The 49-day yogurt gel made with corn starch (Figure 4C) had more void space and fewer apparent interconnections in the strands making up the gel network compared with 7- day and 28-day yogurts (Figure 4A and Figure 4B). Corn starch molecules appeared not to integrate onto the casein micelle network (Figure 4A, Figure 4B, and Figure 4C). The amount of starter culture bacteria increased as storage time increased, which is typical for cultured yogurt products and was to be expected.

It was agreed that increased syneresis with storage time is usually associated with severe casein

network rearrangements (Aguirre-Mandujano *et al.*, 2009). Ramirez-Santiago *et al.* (2010) reported that protein network rearrangements at enriched stirred yogurt by SEM microstructure.

The addition of starch caused the change of microstructure of the yogurt compared to control. Photos of the microstructure suggested that kudzu starch had coarser compact microstructure than yogurt without starch (Data figure is not showed). Yogurt with corn starch showed a relatively loose structure, with the casein micelles linked in chains, with a large number of interspaced voids of varying dimensions as corn starch concentrations increased. The corn starch molecules appeared large and individual molecules within dense protein aggregates comparatively with kudzu starch molecules in yogurt. Kudzu starch (2%) yogurt microstructure exhibited more interconnected clusters of protein particles and kudzu starch molecules surrounded by casein micelle whereas corn starch granules embedded in micelle protein structure. Khalifa and Ibrahim (2015) studied textural properties of camel's milk yoghurt affected by the addition modified starches. Similarly with this study, they found that the images of scanning electron microscopy (SEM) showing that the starch occupied the void space within casein particle network. Conventional milk yoghurts with kudzu starch had more smoothly distributed proteins with a bit coarse structure as well as less porosity in protein network. Yoghurt with 2% kudzu starch gained the higher sensory score compared to the corresponding treatments. The micrographs revealed that images with 2% kudzu starch (Figure 3) had a sponge-like texture with a non-uniform structure. This sponge-like texture could possibly lead to a smoother texture in response to hand-feel and mouth-feel sensory properties of yogurt.

## Conclusion

The result of present study suggest that kudzu starch could be used as an alternative functional starch as a thickener in yogurt by providing a good texture. Yogurt with 2% starch concentration is recommended to stabilize the texture without affecting the flavour of the final product. In addition, the existence of daidzein and daidzin in the kudzu starch and the unique characteristics of this starch would be helpful for food application to improve the functionality of the yogurt products.

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