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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Technological Properties of Xanthan Gums Obtained from Waste Bread Using as a Carbon Source and Performance in Pudding as Model Food

Karbon Kaynağı Olarak Atık Ekmeklerin Kullanılmasıyla Elde Edilen Ksantan Gamların Teknolojik Özellikleri ve Model Gıda Olarak Pudingteki Performansı

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Abstract

Technological properties of aqueous solutions of xanthan gums produced by various *Xanthomonas* species using waste bread (WB) hydrolyzate as a carbon source to reduce the overall product cost and to utilize waste bread were investigated and compared with commercial xanthan gum. While the highest water holding capacity was detected in the commercial xanthan gum, oil holding capacity was higher in xanthan gums from *X. campestris* DSM 19000 and *X. axonopodis* pv. *begoniae* than commercial gum. Pudding samples were prepared by the gums obtained and Ostwald de Waele model was successfully described the flow behavior. The highest consistency coefficient (K) value were obtained by the sample without gum addition as 161.2 Pa.s^n, this was followed by the sample with the gum from *X. axonopodis* pv.*begoniae* with 139.3 Pa.s^n and *X. hortorum* pv.*pelargonii* with 133.2 Pa.s^n . Flow behavior index (n) values varied between 0.12 and 0.49 and increased with the addition of the gum. Therefore, this study showed that the pudding samples prepared with the gums from *X. axonopodis* pv. *begoniae* and *X. hortorum* pv. *pelargonii* isolates using waste bread as substrate were found to be more resistant to shear rate and had a more robust gel structure.

Keywords: xanthan, waste bread, *Xanthomonas* species, rheology, pudding, technological properties

Öz

Atık ekmekleri değerlendirmek ve toplam ürün maliyetini azaltmak için karbon kaynağı olarak atık ekmek hidrolizatının kullanılmasıyla çeşitli *Xanthomonas* türleri tarafından üretilen ksantan gamların sulu çözeltilerinin teknolojik özellikleri incenlenmiş ve ticari ksantan gam ile karşılaştırılmıştır. E n yüksek su tutma kapasitesi ticari ksantan gamda tespit edilirken, yağ tutma kapasitesinin *X. campestris* DSM 19000 and *X. axonopodis* pv. *begoniae* tarafından üretilen gamlarda ticari gamdan daha yüksek olduğu saptanmıştır. Üretilen gamlarla puding örnekleri hazırlanmıştır ve Ostwald de Waele modeline göre pudinglerin akış davranışı başarıyla tanımlanmıştır. En yüksek kıvam katsayısı (K) değeri gam ilave edilmeyen örneklerde 161.2 Pa.s^n olarak elde edilmiş olup bunu sırasıyla *X. axonopodis* pv.*begoniae* ve *X. hortorum* pv.*pelargonii* tarafından üretilen gamların ilave edildiği örnekler 139.3 Pa.s^n ve 133.2 Pa.s^n değerleri ile takip etmiştir. Akış davranış indeksi (n) değerleri 0,12 ile 0,49 arasında değişmiş olup gam ilavesi ile artmıştır. Sonuç olarak bu çalışma, *X. axonopodis*pv. *begoniae* ve *X. hortorum* pv. *pelargonii* izolatları tarafından, substrat olarak atık ekmeğin kullanılmasıyla üretilen gamlarla hazırlanan puding örneklerinin kayma hızına daha dayanıklı olduğu ve bu örneklerin daha sağlam bir jel yapısına sahip olduğunu göstermiştir.

Anahtar kelimeler: ksantan, atık ekmek, *Xanthomonas* türleri, reoloji, puding, teknolojik özellikler

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Extended Summary

Gums, which are high-molecular-weight hydrophilic biopolymers, are mostly used as texturizing agents owing to their capability to bind large volumes of water. This feature gives them the ability to modify the functional and rheological features of a food system. Xanthan gum is one of the most widespread gums and is widely used for its thickening properties in dairy products and dessert gels (Phillips & Williams, 2009, chap. 7).

Xanthan gum, the first natural biopolymer produced at an industrial scale, is a microbial heteropolysaccharide secreted by *Xanthomonas campestris*. Xanthan is an exopolysaccharide and its the primary structure is consisted of complex repeating units of pentasaccharides being composed of two glucose, two mannose and one glucuronic acid residue, along with a pyruvic acid unit. It was first discovered in 1963 and has been fully utilised ever since. (Gilani, Najafpour, Heydarzadeh, & Zare, 2011; Freitas et al., 2015; Palaniraj et al., 2011).

Xanthan gum is industrially produced using commonly glucose or sucrose as the solitary carbon source. However, commercially available xanthan gum is comparatively expensive because of carbon source used in the production and this has obstructed wider applications. The cost of the fermentation medium is a major care in the xanthan manufacturing. Using cost effective, reliable and more plentiful carbon sources, such as waste bread, for commercial xanthan production can reduce the production cost and, consequently, improve the economy of the process (Yoo and Harcum 1999).

Xanthan gum possesses significant properties such as good thickening agent, emulsification, stability, pseudoplasticity, temperature resistance, water solubility and excellent compatibility which make it an important commodity for use in food, paper, paint, cosmetic and pharmaceutical industries (Sanderson, 1981). Xanthan is used in food industries as thickener, emulsifier and stabilizer owing to its reological features such as high viscosity and pseudo-elasticity (Garcia-Ochoa et al. 2000; Plank 2004). The gum presents many superiorities as a thickener, stabilizer, gelling and texturizing agent and suspending agent, is commonly used for these features in dairy products, processed meats, chicken or fish, creams, synthetic juices, salad dressing, syrups and coverings for ice creams and desserts. Even use of xathan at low concentration in food products gives highly viscous solution.(Luvielmo & Scamparini, 2009; Nussinovitch, 1997; Casas et al. 2000). These properties of xanthan gum provide great advantage for use in products such as puddings.

Puddings can be defined as milk-based starch pastes and have a characteristic semisolid food texture whose rheological properties are in between a gel and a fluid (Lim and Narsimhan 2006). Their texture are usually formed suspension of deformable particles (the swollen starch granules) dispersed in a continuous medium containing milk proteins as well as a stabilizer (Doublier & Durand, 2008). Although starch act an important part in the properties of the system and the development of body and mouthfeel of a pudding matrix; the interactions among substances such as milk components, sugar and gums also remarkably modify the microstructure of the system and causes a clear increase in viscosity (Vélez-Ruiz et al., 2006).

The objective of this work was to determine technological properties of xanthan gums obtained from waste bread using as a carbon source. Moreover, our aim was to make rheological analyses of pudding samples and also to evaluate the gum performance in the food samples produced by gums.

Materials and Methods

Raw Materials

Stale wheat bread that was run out of shelf life was obtained from a bakery. Enzymatic hydrolysis of waste bread was carried in optimum conditions found by Demirci et al. (2017a). The same materials were used for hydrolysis as those used in Demirci et al. (2017a). Consequently, glucose which was obtained from enzymatic hydrolysis of waste bread was used for xanthan synthesis by the studied bacteria. All the ingredients used for pudding manufacture (with the exception of deionized water and gums) were commercial products, purchased at a local market.

Microorganism, Medium and Cultivation Conditions

X. campestris DSM 19000 (NRRL B-1459), *X. axonopodis* pv. *vesicatoria*, *X. hortorum* pv. *pelargonii* and *X. axonopodis* pv. *begoniae* were used to produce xanthan gum from waste bread. Mediums and cultivation conditions, xanthan production, recovery and purification were described in Demirci et al. (2017b).

For each microorganism, production was carried out under fermentation conditions in which obtained gums exhibited the highest viscosity (data not shown as this is not the scope of this work). The fermentation conditions which were determined in Demirci et al. (2019) are shown in Table 1.

Table 1. Fermentation conditions which production was carried out (Demirci et al., 2019)

	glucose ratio (%)	inoculum volume (%)	mixing rate (rpm)
X. hortorum pv. *pelargonii*	4	5	175
X. axonopodis pv. *begoniae*	4	5	250
X. axonopodis pv. *vesicatoria*	4	5.5	300
X. campestris DSM 19000	4	5	225

Determination of Technological Properties

Water holding capacity

The method of Johnson (1970) was used for detection water holding capacity (WHC) of xanthan gums. For this purpose 1 g of xanthan gum was added to 10 mL distilled water, suspended in it, then vortexed for 2 min and then centrifuged at 3000 x g for 30 min. The water absorbed by the samples was expressed as grams of water absorbed per 100 g of xanthan gum after decanting free water.

Oil holding capacity

Similarly the method of Johnson (1970) was used for determining the oil holding capacity of xanthan gums. 1 g of xanthan gum was added to 10 mL of refined sunflower oil and dispersed in it, then vortexed for 2 min and then centrifuged at 3000 x g for 30 min. The oil holding capacity was demonstrated as grams of oil absorbed per 100 g of xanthan gum

Solubility measurements

0.1% w w^{-1} xanthan gum solution was stirred at 25 ° C for 30 min with magnetic stirrer and the solution was centrifuged (6000g, 30 min, at 20 °C) to remove the insoluble substance. The supernatant was removed, dried at 105 ° C for 24 hours and the supernatant concentration determined (Dakia et al. 2008).

$$=\text{solubility (\%)} = \frac{\text{supernatent conc (mg=ml)}}{\text{initial preparation conc (mg=ml)}} x100 \qquad (1)$$

Preparation of the pudding samples

Pudding samples were prepared using 250 ml whole fat milk (3% fat) 6.25 g starch, 37.5 g sucrose and 0.5 g xanthan gum. The dry ingredients were mixed with milk in a magnetic stirrer for 1 hour at room temperature. The mixture was heated to boiling for 3 minutes and then added to the containers and cooled to 4 ° C. Prepared pudding samples were stored at 4°C for 24 hours and then rheological analyzes were performed.

Rheological analyses

The rheological measurements of pudding samples were conducted with stress-controlled rheometer (TA DHR-2, USA) at 4 °C. Steady shear measurements were carried out with a parallel plate (diameter 40 mm) between shear rate of 1-100 s^{-1} and performed in duplicate. Power-law (Ostwald de Waele) was used to model rheological data and the determinant coefficient (R^2), consistency coefficient (K) and flow behavior index (n) values were detected (Eq. 2).

$$\sigma = K(\dot{\gamma})^n \qquad (2)$$

where, σ is shear stress (Pa) and γ is shear rate (s^{-1}).

Dynamic oscillatory tests were conducted by frequency sweep measurements within the linear viscoelastic region. Frequency sweep test was conducted at 0.6 Pa between frequency range of 0.05–100 rad s^{-1}. The elastic or storage modulus (G') (Eq. 3) and the viscous or loss modulus (G") (Eq. 4) were modeled by a power law;

$$G' = K'(\omega)^{n'} \qquad (3)$$
$$G'' = K''(\omega)^{n''} \qquad (4)$$

where ω is angular frequency (rad s^{-1}), K' and K" were intercepts, n' and n" were elastic or viscous behavior index, respectively.

Results and Discussion

Technological Properties of Xanthan Gums Produced From Waste Bread

Water and oil holding capacities

The water and oil holding capacities of the gums produced by the three isolates and standard species used in our study under the highest viscosity conditions are shown in Table 2.The values obtained were compared with the commercial xanthan gum. The amount of water absorbed by 100 g gum was found to be the highest in the commercial gum compared to other gums. The highest water holding capacity was obtained by the gum from *X. axonopodis*pv. *vesicatoria* and followed by the gums from *X. campestris* DSM 19000, *X. axonopodis* pv. *begoniae, X. hortorum* pv. *pelargonii.*

The amount of oil absorbed by 100 g produced gum from *X. hortorum*pv. *pelargonii, X. axonopodis* pv. *begoniae, X. axonopodis* pv. *vesicatoria, X. campestris* DSM 19000 and commercial gum were found to be 104, 112, 90, 156 and 107 g, respectively. When all gums are compared, the highest oil holding capacity was obtained by the gum from *X. campestris*DSM 19000. While the highest water holding capacity was detected in the commercial xanthan gum, oil holding capacity was higher in xanthan gums from *X. campestris* DSM 19000 and *X. axonopodis* pv. *begoniae* than commercial gum.

Table 2. Water and Oil Holding Capacity of Xanthan Gum Samples

	Amount of water absorbed by 100 grams of gum (gr)	Amount of oil absorbed by 100 grams of gum (gr)
X. hortorum pv. *pelargonii*	210	104
X. axonopodis pv. *begoniae*	540	112
X. axonopodis pv. *vesicatoria*	710	90
X. campestris DSM 19000	410	156
Commercial gum	1240	107

Solubility measurements

The solubility values of the gums produced by the three isolates and standard species under the highest viscosity conditions are shown in Table 3. The values obtained were compared with the commercial xanthan gum. The solubility values of xanthan gums from highest to lowest values were ordered as; *X. campestris* DSM 19000, *X. hortorum* pv. *pelargonii*, commercial gum, *X. axonopodis* pv. *vesicatoria and X. axonopodis* pv. *begoniae.* Difference in solubility and water/oil holding capacities of xanthan gums may be resulted from the variance in chemical structures and H-bonding forming sites as particular isolates could produce gums having different mannose and glucuronic acid contents.

Table 3. Solubility values of Xanthan gum samples

	Solubility (%)
X. hortorum pv. *pelargonii*	0.0010352
X. axonopodis pv. *begoniae*	0.00062565
X. axonopodis pv. *vesicatoria*	0.00062591
X. campestris DSM 19000	0.00104756
Commercial gum	0.00083368

Rheological Characterization of Pudding Samples

Rheograms of pudding samples prepared with the xanthan gums were shown in Fig. 1

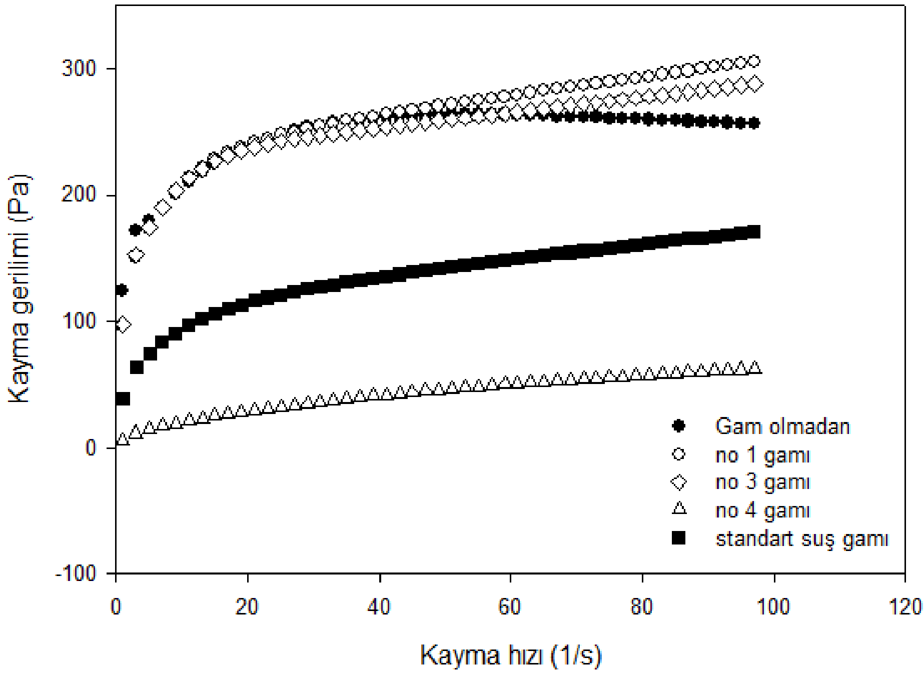

Figure 1. Shear stress versus shear rate data of pudding samples between 1-100 s^{-1} (sample 1-*X. hortorum* pv. *pelargonii*, sample 2-*X. axonopodis*pv.*begoniae*, sample 3-*X. axonopodis*pv.*vesicatoria*, standard-*X. campestris* DSM 19000).

In general, pudding samples showed shear thinning flow behavior similar to gum-water solutions in accordance with literature (Doublier and Durand, 2008). As shown in Figure 1, the samples prepared with gums produced from *X. hortorum* pv. *pelargonii*and *X. axonopodis* pv. *begoniae* isolates and the samples without adding gums showed similar flow behavior and their viscosities were found higher than pudding samples prepared with gum obtained from *X. campestris* DSM 1900 standard strain and *X. axonopodis* pv. *vesicatori a*isolates. In the sample without adding gum, the shear stress decreased as the shear rate was increased from 50 s^{-1} and this is an indication that the structure is more easily damaged than the samples with gum. The shear stress data versus shear rate parameters obtained from the adaptation to the Ostwald de Waele model are given in Table 3.

Table 4. The effect of Xanthan gums produced from different bacteria and waste bread on ostwald de waele of pudding samples at 20 °C.

Samples	*K* (Pa s^n)	*n* (-)	R^2
X. hortorum pv. *pelargonii*	133.2	0.18	0.97
X. axonopodis pv. *begoniae*	139.3	0.16	0.94
X. axonopodis pv. *vesicatoria*	6.5	0.49	0.99
X. campestris DSM 19000	5.0	0.27	0.99
Without gum addition	161.2	0.12	0.83

R^2value between 0.83 and 0.99 showed that the model is suitable for flow behavior. Consistency index (K) decreased when xanthan gum was added to pudding samples, the highest decrease was observed when the gums produced from *X. axonopodis*pv. *vesicatoria* and *X. campestris* DSM 1900 strains were added. In these examples, the K value was strikingly 6.5 and 5 Pa.s^n respectively. This was followed by *X. hortorum* pv.*pelargonii*with 133.2 Pa.s^n and *X. axonopodis* pv.*begoniae* with 139.3 Pa.s^n. K value was found to be 161.2 Pa.s^n in the sample without gum addition. The reason why K values decreased with the addition of gum in pudding samples might be the presence of antagonistic effect between starch and produced xanthan gums on viscosity. n values varied between

0.12 and 0.49 and increased with the addition of the gum. It was observed that a robust structure which can withstand the shear rate more was gained.

Considering dynamic oscillation analysis of pudding samples (Figure 2), G' value was found higher than G" value in all samples. It was determined that elasticity predominates over liquid property in pudding samples, pudding samples showed weak gel properties. The pudding samples prepared with the gums obtained from *X. hortorum* pv. *pelargonii* and *X. axonopodis* pv. *begoniae* isolates and without gum had the highest G'. On the other hand, the pudding samples prepared with the gums obtained from *X. axonopodis* pv. *vesicatoria* isolate had the lowest G'. By adapting the obtained G 'and G" data to the Power-law model (Table 4), the highest K 'value was found to be in the pudding sample without gum addition. This was followed by K 'values of pudding samples prepared with the gums obtained from *X. axonopodis*pv. *begoniae, X. hortorum* pv. *pelargonii*, *X. campestris* DSM 19000 and *X. axonopodis* pv. *vesicatoria isolates*, respectively.

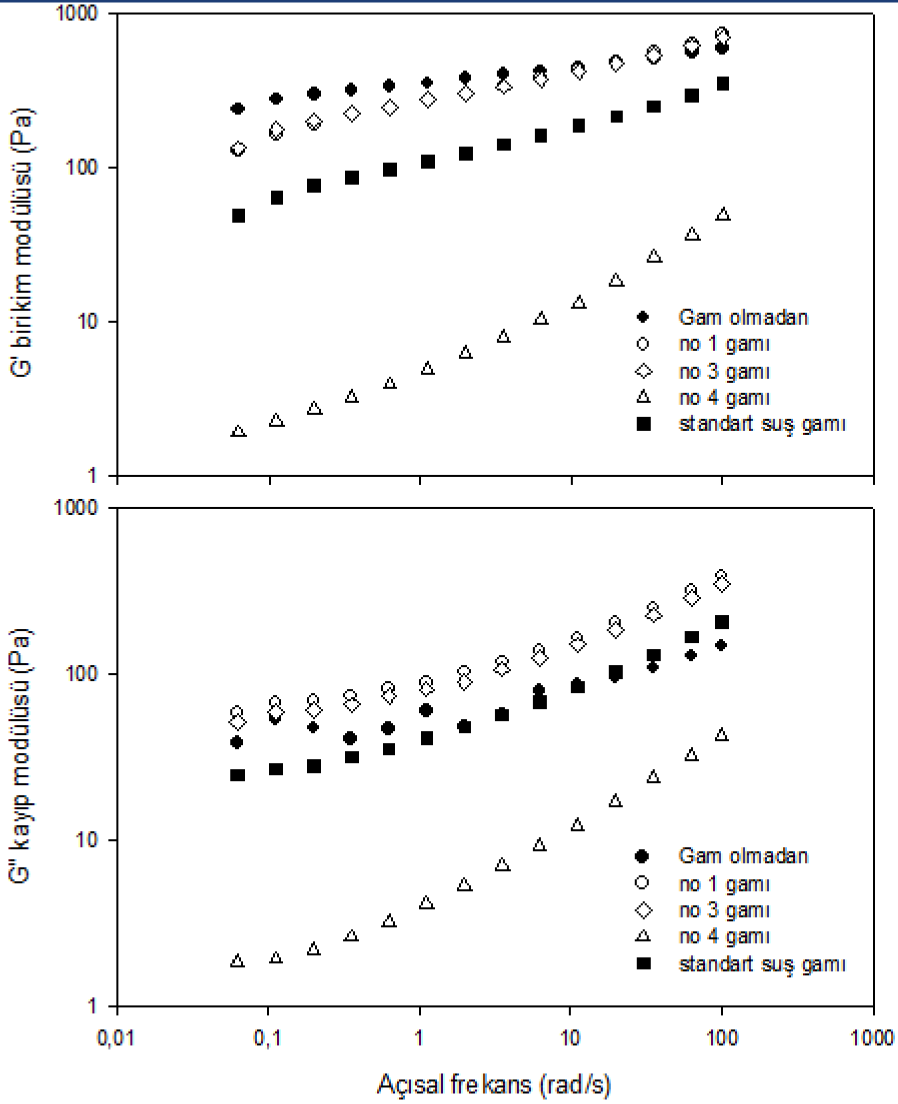

Figure 2.G' and G" Values versus angular frequency data of pudding samples between 0,05-100 rad s^{-1} (sample 1-*X. hortorum* pv. *pelargonii*, sample 2-*X. axonopodis* pv.*begoniae*, sample 3-*X. axonopodis*pv.*vesicatoria*, standard-*X. campestris* DSM 19000).

Table 5. The eect of Xanthan gums produced from different bacteria and waste bread on loss modulus and storage modulus parameters of pudding samples at 20 °C.

Samples	*G'*			*G''*		
	K'	*n'*	*R²*	*K''*	*n''*	*R²*
X. hortorum pv. *pelargonii*	255.3	0.22	0.99	84.6	0.32	0.98
X. axonopodis pv. *begoniae*	259.8	0.2	0.99	75.7	0.32	0.98
X. axonopodis pv. *vesicatoria*	3.67	0.55	0.99	3.47	0.54	0.99
X. campestris DSM 19000	103	0.25	0.99	37.5	0.36	0.99
Without gum addition	343.6	0.11	0.99	53.7	0.2	0.97

G ': storage modulus; G ": loss modulus; R^2*:* determination coefficients

The most important result from rheological analysis of pudding model food; although viscosity and viscoelastic properties of sample without gum addition were better than gum-added samples, the puddings prepared with the gums obtained from *X. axonopodis* pv. *begoniae*ve *X. hortorum* pv. *pelargonii* isolates using waste bread as substrate were found to be more resistant to cutting speed and angular frequency and have a more robust gel structure.

Conclusion

The development of xanthan gum producing microorganisms with high yield, supplying inexpensive substrates are now essential to produce xanthan gum biotechnologically at a lower price. In this study, xanthan gums were successfully obtained from four different strains by using waste bread as a carbon source. This work showed that the xanthan gum produced from waste bread hydrolyzate had similar technological properties to the commercial xanthan gum. The water and oil holding capacity and solubility results were observed between the commercial xanthan gum and gums produced from waste bread. Pudding sample as model food was prepared by the gums obtained, moreover the pudding samples produced were found to be more resistant to physical damage and had a more robust gel structure. The starch–xanthan gum combinations have potency to be widely used as stabilizer and thickener with various different industrial applications in food industry.

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