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The Impact of *Cassia fistula* Polysaccharide on Flavor Compounds Formation and the Rheological Properties of Yogurt during Storage

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Darwish, M. S.*; M. Sh. Gomaa and Noura A. Haider

Dairy Department, Faculty of Agriculture, Mansoura University, Mansoura 35516, Egypt.

ABSTRACT

The effect of adding *Cassia fistula* polysaccharide (CFP) at levels of 1%, 1.5%, or 3% (w/w) on the physicochemical properties of natural yogurt was analyzed. The physical stability (wheying-off and syneresis), texture parameter (hardness, adhesiveness, cohesiveness, springiness, gumminess, chewiness) and flavor compounds formation (acetaldehyde and diacetyl) were investigated. Yogurt enriched with CFP showed significantly lower wheying-off and syneresis compared to the control, with 2% CFP having the lowest values. Hardness decreased as CFP concentration and storage time at 4°C increased. Adhesiveness was also lower in CFP-enriched samples but increased during storage. Adding 2% CFP reduced consistency, cohesiveness, and springiness, indicating a softer texture. Gumminess and chewiness were lower in CFP-enriched samples, with 2% CFP yogurt showing the lowest gumminess (0.31 N). Acetaldehyde levels in CFP-enriched yogurt were lower than the control and decreased over time, while diacetyl levels increased during storage but remained lower than in the control.

Keywords: Adhesiveness; cohesiveness; hardness; polysaccharides; springiness; syneresis

INTRODUCTION

Sociocultural influences, lifestyle decisions, and eating patterns have increasingly become vital components in supporting health (Wang *et al*., 2023). According to the Global Burden of Disease Study 2017, poor diets were responsible for approximately 11 million deaths worldwide (Li *et al*., 2021). Consequently, enhancing nutritional quality and dietary patterns could aid in managing and preventing chronic non-communicable diseases while reducing mortality rates.

Fermented foods, whether non-dairy (such as juices, vegetables, fruit products, cereals, and meat) or dairy (like yogurt, ice cream, cheese, cream, and fermented milk), are considered excellent examples of functional foods due to their bioactive compounds and other nutrient-dense components, such as vitamins, antioxidants, minerals, and organic acids.

There is a growing and continuous trend in consumer interest and awareness regarding proper nutrition. Functional foods, which are promoted for their nutritional and health benefits, are gaining more popularity (Darwish *et al*., 2022a; Darwish *et al*., 2022b; Darwish *et al*., 2023a; Darwish *et al*., 2023c; Darwish *et al*., 2024). Considering the health claims, a connection between food and health must be established, backed by scientific and clinical studies (El Dessouky Abdel-Aziz *et al*., 2020; Elbermawi *et al*., 2022a; Elbermawi *et al*., 2022b; Khojah *et al*., 2022). Functional foods either include components that have a beneficial impact on health or remove components with harmful effects. They also contain bioactive substances, such as dietary fiber (Homayouni *et al*., 2012).

In this regard, yogurt consumption is associated with its health advantages and nutrient-rich composition (Huang *et al*., 2020). However, a common technical issue arises during fermentation when caseins reach their pH 4.6, leading to the micelles formation. These micelles then aggregate with whey proteins, resulting in the protein gels creation. However, these pure gels are more susceptible to syneresis and have lower stability (Xu *et al*., 2019). To address incorporating additives can improve the stability and texture of yogurt, extending its shelf life while maintaining its sensory qualities.

In recent years, there has been a strong push for bioprospecting biological products as alternatives to artificial ingredients that are now on the market. Utilizing raw materials, primarily organic macromolecules, provides a basis for developing sustainable and bioactive products (Albuquerque *et al*., 2022). Biopolymers, large molecules made up of different monomer units like proteins, polysaccharides, polyesters, and polyphenols, are derived from natural resources and suggested for various biotechnological uses (Albuquerque *et al*., 2022).

Polymers derived from algae, bacteria, plants, animals, and fungal fermentation are called polysaccharides. They are preferred over synthetic polymers because of their low or non-toxicity, biodegradability, sustainability, and biocompatibility. These characteristics have made polysaccharides popular biopolymers in a variety of industries for a long time (Albuquerque *et al*., 2022). They can be categorized as cationic, anionic, or neutral depending on their electrical charge. Furthermore, factors like temperature, pH, molecules concentration and ionic strength during the extraction process affect the structure of the main polysaccharide chain.

Polysaccharides extracted from *Cassia fistula* are noteworthy, as they are classified as soluble fiber due to their resistance to hydrolysis in the human digestive system and their prebiotic properties (Dawood *et al*., 2021). Additionally, these polysaccharides possess various functional characteristics, including strong gel-forming

* **Corresponding author.**
E-mail address: msamir@mans.edu.eg
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ability. Notably, one of their key features is their fat-mimicking property, which has been effectively utilized to improve the production of functional yogurt. This paper aims to utilize texture profile analysis to identify and assess the extent of physical changes in natural yogurt with the inclusion of a prebiotic. The study's objective was to evaluate the impact of adding polysaccharides extracted from *Cassia fistula* as a functional ingredient on the characteristics of natural yogurt.

MATERIALS AND METHODS

Materials

In Mansoura, Egypt, mature fruits of the *Cassia fistula* were harvested from the campus of Mansoura University. All the chemicals utilized in the study were acquired from Sigma-Aldrich, whereas the starter culture was obtained from Chr. Hansen.

The standardized bovine milk, obtained from the local market in Mansoura City, contained 3% milk fat, 12 total solids, 3.5% protein and had a pH of 6.68.

Crude polysaccharide extraction process

The crude polysaccharide was produced following the procedure outlined in the study conducted by Dawood *et al*. (2021).

Preparation of yogurt enriched with varying concentrations of CFP

Yogurt enriched with varying concentrations of CFP (1%, 1.5%, and 2%) was prepared following the method described by Darwish *et al.* (2023b) with some modifications, Four yogurt formulations were prepared using the process outlined in Figure 1: a control (without CFP), yogurt with 1.0% CFP, yogurt with 1.5% CFP, and yogurt with 2% CFP.

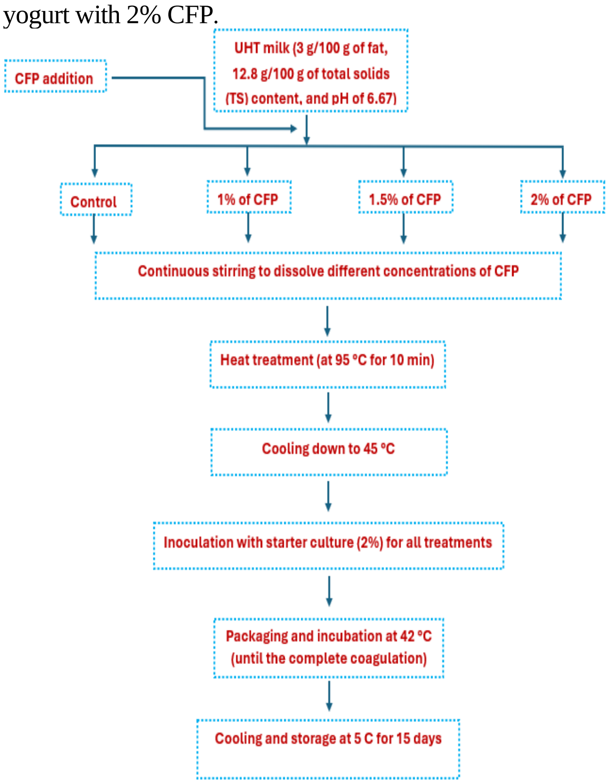

Figure 1. Yogurt production flowchart: Control refers to yogurt without CFP, 1% CFP is yogurt with 1% CFP, 1.5% CFP is yogurt with 1.5% CFP, and 2% CFP is yogurt with 2% CFP.

Physical properties

Wheying-off:

Whey separation from yogurt without applying any forces, using the siphon technique outlined by Amatayakul *et al*. (2006).

Syneresis

The syneresis of yogurt was assessed using a modified version of the method outlined by Amatayakul *et al.* (2006).

Texture profile analysis

With the use of a texture profile analyzer (TA 1000, Lab Pro (FRC TMS-Pro), USA), the texture characteristics of yogurt were evaluated. A cylinder probe with a 25 mm diameter was used for the analysis and the crosshead speed of 50 mm/s. Parameters such as adhesiveness, hardness, springiness, cohesiveness and chewiness were measured in triplicate, following the method described by Bourne (1978).

Measurement of acetaldehyde and diacetyl

Using a Jenway UV/visible spectrophotometer, the amounts of acetaldehyde and diacetyl in yogurt samples were determined in accordance with the procedure outlined by Lees and Jago (1970).

Statistical Analysis

Each experiment was conducted in triplicate. An ANOVA test, with a significance threshold of $p < 0.05$, was used to examine changes in Wheying-off, Syneresis, Hardness, Adhesiveness, Cohesiveness, Springiness, Gumminess, Chewiness, Acetaldehyde, and Diacetyl properties. The results were presented as mean ± standard deviation. Significant differences between the values were determined using Duncan's multiple range test.

RESULTS AND DISCUSSION

Physical properties of yogurt

It is often recognized that the structure of fermented milk products greatly influences several aspects such as functionality, texture, and appearance. The microstructure and structural layout of the protein interaction network determine the yogurt's texture and rheological properties (Delikanli and Ozcan, 2017). Figures 3, 4, 5, 6, and 7 show the texture profile analysis (TPA) characteristics (adhesiveness, hardness, cohesiveness, springiness, chewiness, and gumminess) of the yogurt samples that were examined.

Wheying-off evaluation of yogurt:

Whey separation in yogurt is undesirable and can happen when the body is weak. Whey-off is the process of separating whey from the gel without the need for an outside force. Yogurt with varying levels of CFPs had a considerably ($p > 0.05$) lower wheying-off percentage than the control. The yogurt sample with 2% CFPs had the least amount of whey separated out of all of them; this was likely due to the greater exopolysaccharide concentration. As the storage time progressed, there was a considerable rise in wheying-off (Figure 2a). It was found that low-fat yogurt containing EPS exhibited comparable wheying-off behavior during storage (Ramchandran and Shah, 2010). These findings are in line with those of Hassan *et al*. (2015), who found that yogurt samples with varying concentrations of guar gum (GG) or cress seed mucilage (CM) showed less wheying-off when compared to the control group.

Syneresis evaluation of yogurt

Figure 2b illustrates the syneresis of yogurt samples with varying levels of CFPs. When compared to the other treatments, the sample with 2% of CFPs had the greatest exopolysaccharide content, which was associated with the lowest whey separation (7.89%). As the storage period extended, there was a notable rise in syneresis. It was observed that a greater exopolysaccharide concentration contributes to reduced whey separation. Many studies by other authors support the findings of the current study (Nikoofar *et al*., 2013; Hassan *et al*., 2015). The findings of the current study may be attributed to the exopolysaccharide's high water-binding capacity and its ability to modify the yogurt's microstructure through its content (Nikoofar *et al*., 2013; Hassan *et al*., 2015).

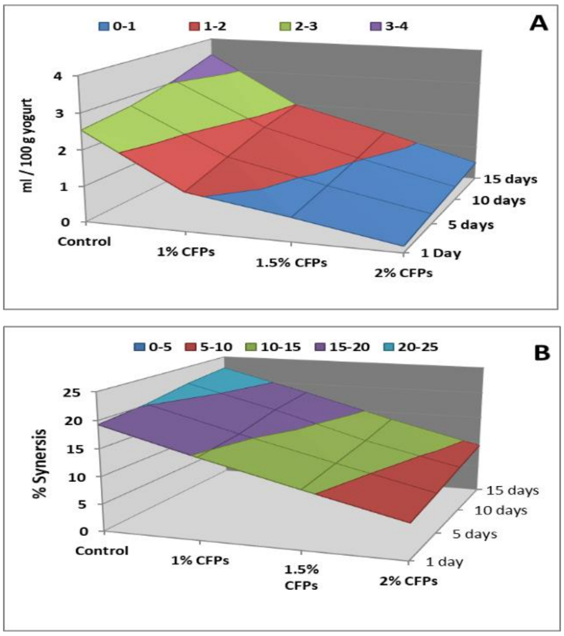

Figure 2. The effect of storage time and CFP content on wheying off (a) and syneresis (b).

Hardness evaluation of yogurt

Hardness, or firmness, is the parameter most often evaluated for texture profile analysis of yogurt that is was known as the essential strength required for sample deformation (Mudgil *et al*., 2017). The values of hardness were gathered during storage periods. Hardness significantly increased with storage (Figure 3a). Hardness values of yogurt fortified with different concentration of CFPs ranged from 0.94 to 1.6 N (Fig. 4c). The CFPs enriched yogurt samples have significantly lower hardness values than control. The hardness degree of experimented samples is inversely proportional with concentration of CFPs and storage time of samples at 4°C. Until the conclusion of the storage periods (15 days), all examined samples had considerably higher hardness levels ($p < 0.05$).

When the values of hardness are compared with the previous studies, findings comparable to our results were determined by Kose *et al.* (2008) in addition to Wen *et al.* (2014). However our hardness values greater than those reported by Karahan (2016). The amount of culture, incubation temperature, and duration have all been implicated in these variations. Accordingly, (Mudgil *et al*., 2017) found that the hardness values are closely correlated with the culture level utilized to produce yogurt, with the maximum degree of hardness in the examined samples being between 2-2.5% of the starter culture percentage. Furthermore, it was reported by (Sah *et al*., 2016) that a shorter incubation period has also been linked to yogurt with lower hardness levels. Particularly, shorter incubation period of yogurt may adversely impact the textural characteristics of yogurt. A faster rate of acidification causes the casein micelles' colloidal calcium phosphates to be depleted, releasing individual caseins from the micelles and causing a casein network to develop. In this case, fast casein coagulation leads to highly rearrange particles and small number formation of protein-protein bonds, leading to weak gel formation with larger pores, which in turn causes increased whey syneresis (Sah *et al*., 2016). Another study by Lee and Lucey (2003) found that lower incubation temperatures result in a stronger protein network and increased gel hardness. These findings align with those of Azari-Anpar *et al.* (2017) , who reported that Aloe Vera gel-enriched yogurt reduced the hardness values of the tested samples. The lowest hardness value was observed in the sample enriched with 5% *Aloe vera* gel, which may be due to the salicylic acid in the gel inhibiting the growth of the yogurt starter culture. Michael *et al.* (2010) also demonstrated that plant extracts, such as onion, olive, garlic, and citrus, reduce yogurt hardness. However Mudgil *et al.* (2017) found that fortifying yogurt with 2-2.5% partially hydrolyzed guar gum (PHGG) had no effect on hardness, while adding more than 2.5% reduced hardness. Additionally, Helal *et al.* (2018) reported that adding inulin to yogurt significantly decreased ($P < 0.05$) its hardness, with the lowest value observed in the sample enriched with 3% inulin.

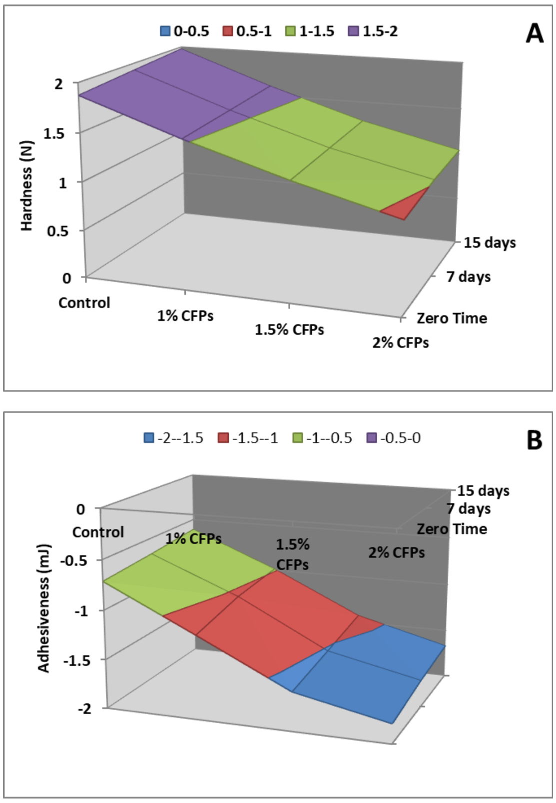

Figure 3. The influence of varying level of CFPs and time of storage on hardness (a) and adhesiveness (b).

Adhesiveness evaluation of yogurt

The energy required to overcome the adhesion force between a sample and other surfaces was known as adhesiveness. The adhesiveness values characterized negative sign meant force pointed down. A negative stress is generated due to remove male probe from the tested samples. Adhesiveness, a key texture property of yogurt, has been found to positively impact its thickness and is also used as a criterion for assessing yogurt's stability during storage (Helal *et al*., 2018). Adhesiveness values of yogurt fortified with different concentration of CFPs ranged from -0.98 to -1.8 mJ (Figure 3b). The CFPs enriched yogurt samples have significantly ($P<0.05$) lower adhesiveness values than control. The adhesiveness of all treatments increased during storage at 4°C (Figure 3b).

The current results are consistent with previous studies, which reported that increasing the concentration of inulin leads to a more significant reduction in adhesiveness. The sample containing 3% inulin exhibited the lowest adhesiveness value (Helal *et al*., 2018). Similar observation were reported by Tavakolipour *et al*. (2014), who found that higher levels of fat replacers, such as waxy corn starch and gelatin, were associated with a reduction in adhesiveness. This effect is likely due to the increased concentration of hydrocolloids, which promotes the formation of a weaker three-dimensional protein network. It was common knowledge that polysaccharide-protein interaction and the behavior gelation and aggregation have important effect in physical stability, structure and rheological characteristics of multicomponent food systems (Dickinson, 2008; Semenova *et al*., 2009; Nagae and Yamaguchi, 2014; Benbettaïeb *et al*., 2016). When the solution's pH is near the protein's isoelectric point (pI), the likelihood of forming a weak protein network from protein-polysaccharide interactions is higher compared to when the pH is lower than the pI (Turgeon *et al*., 2007; Dickinson, 2008). The incorporation of inulin into yogurt negatively impacts its adhesiveness because the pH in various yogurt treatments is close to the protein's isoelectric point (pI).

Dickinson has also presented that biopolymers concentration is significantly impacting on the rheological properties of food matrix (Dickinson, 2011). The using high inulin concentration which having identical charge resulting in weak protein network even in protein pI lower than pH due to net repulsion which provide high ionic strength (Grinberg and Tolstoguzov, 1997). In general, the adhesiveness of yogurt increased up to the 14th day of storage, although the rate of increase varied depending on the treatment. The control sample exhibited the highest adhesiveness values during storage, with measurements of -0.71±, -0.67±, and -0.61± mJ for days 0, 7, and 15, respectively. Kip *et al*. (2006) and Abou-Soliman *et al*. (2017), observed similar results, noting an increase in yogurt adhesiveness during storage, followed by a decrease at the end of the storage period.

Cohesiveness evaluation of yogurt

Consistency, or cohesiveness, is an important textural attribute of yogurt and reflects its acceptance from the consumer's perspective. Consistency indicates the strength of internal bonds, which making up the food body as a perfect, and it is expressed as the strength degree that may result deformed a food material before it is cracked (Chandra and Shamasundar, 2015). As can be noticed from Figure 4a, the cohesiveness values of the investigated samples were significantly impacted by CFPs ($p < 0.05$). CFPs incorporation (2%) into the samples of yogurt decreased consistency value from 0.45 (control) to 0.34. Low cohesiveness value presented that samples structure containing CFPs is less force and firmer compared with control. The cohesiveness values of yogurt samples are inversely proportional with cold storage (Figure 4a). Our results are consistent with those Azari-Anpar *et al*. (2017), who reported that yogurt enriched with *Aloe Vera* gel is related to decrease in samples cohesiveness. Also, the fortification of yogurt with different concentration of dried grape pomace (3, 4 and 5%) lead to reduce values of cohesiveness in tested samples (Mohamed *et al*., 2014).

Springiness evaluation of yogurt

Springiness (mm), or elasticity, reflects the degree and speed at which a deformed food material returns to its original position after the force is released. Several factors effect on values of yogurt springiness such as protein interaction, heat treatment and protein unfolding degree (Delikanli and Ozcan, 2017). The effect of CFPs addition on yogurt springiness is shown in (Figure 4b). Yogurt springiness scores varied significantly ($p < 0.05$) between the CFP-enriched and control groups. Yogurt samples springiness was presented minimum at level 2% of CFPs and storage time of 15 days (0.60 mm) (Figure 4b). The CFPs addition associated with decreased the yogurt springiness with increasing its percentage. The values of springiness have been significant decreased ($p < 0.01$) until the 15th day of storage time (Figure 4b). Helal *et al*. (2018) found similar results, reporting that adding inulin reduces the springiness of yogurt as its amount rises.

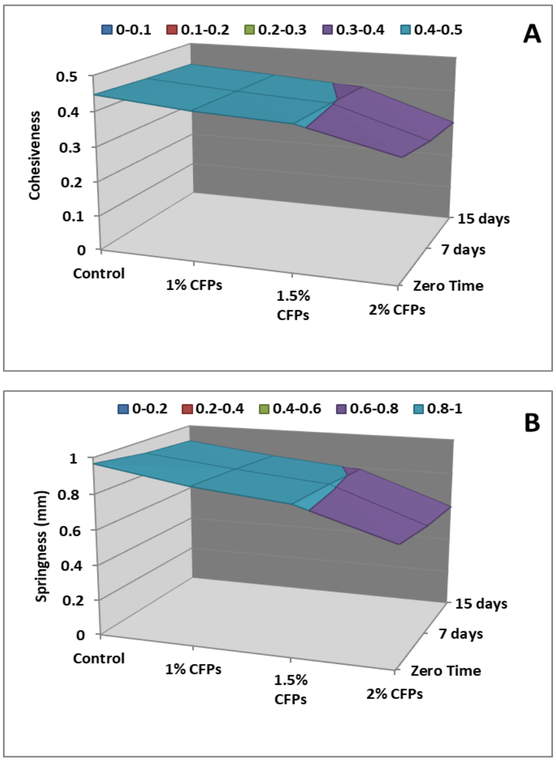

Figure 4. The influence of varying levels of CFPs and storage duration on cohesiveness (a) and springiness (b).

Gumminess evaluation of yogurt

Gumminess refers to the energy required to break down a semisolid food into smaller pieces until it is suitable for swallowing (Domagała *et al*., 2006). The gumminess range was 0.32 to 0.85 N. The highest and lowest value of gumminess was related to control (0.85 N) and yogurt enriched with 2% CFPs (0.31 N) (Figure 5a) respectively. The gumminess values of control and yogurt containing 1 and 1.5% of CFPs increased with prolonged cold storage (Figure 5a), while the yogurt gumminess containing 2% of CFPs decreased with increased cold storage time. Our findings aligned with those of Nikoofar *et al*. (2013), discovered that adding quince seed mucilage to yogurt resulted in a decrease in gumminess.

Chewiness evaluation of yogurt

Chewiness refers to the effort or time needed to chew a food sample until it is ready to be consumed. It is influenced by factors such as hardness, springiness, and cohesiveness. Yogurt samples chewiness ranged from 0.18 to 0.80 J (Figure 5b) according to CFPs concentration and cold storage. The concentration of CFPs has a significantly negative impact on chewiness. The decrease in yogurt chewiness containing CFPs might be attributed to high moisture content in CFPs enriched yogurt macerates the protein interaction network, which fortunately resulted in lower firmness and subsequently decreased other textural parameter of yogurt (Cohesiveness, gumminess and chewiness). Additionally, polysaccharides can interact with the protein network, resulting in a soft and smooth yogurt curd that coats the mouth during chewing (Hassan *et al*., 2003; Hassan *et al*., 2004).

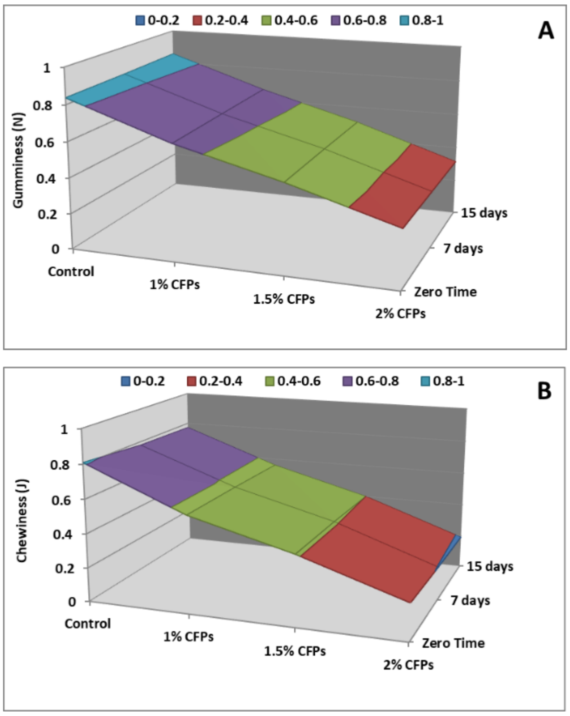

Figure 5. The impact of varying CFP levels and time of storage on gumminess (a) and chewiness (b).

Flavor compounds in yogurt

Acetaldehyde evaluation of yogurt

The variations in volatile flavor compounds of yogurt samples, measured by acetaldehyde concentration, are presented in Figure 6a. The acetaldehyde levels in the yogurt samples ranged from 63.3 to 88.67 µmol/100g, depending on the concentration of CFPs and cold storage conditions (Figure 6a). The acetaldehyde concentration significantly decreased ($p > 0.01$) over the storage period at 4°C. Yogurt enriched with different concentrations of CFPs (1%, 1.5%, and 2%) exhibited lower acetaldehyde levels compared to the control across various storage periods (Figure 6a). The concentration of acetaldehyde in yogurt samples enriched with CFPs significantly decreased ($p < 0.01$) with extended storage time, with the highest levels observed at the beginning (zero time) and the lowest at the end of the 15-day storage period (Figure 6a). The acetaldehyde levels in all treatments remained within the optimal range (23 to 41 mg/kg), which is necessary for producing the standard flavor in yogurt (Yekta and Ansari, 2019). Adding 2% CFPs to yogurt reduces acetaldehyde levels from 36.5 mg/kg (control) to 27 mg/kg. This reduction is beneficial, as acetaldehyde can form from ethanol during the oxidation process and has carcinogenic potential, which could lead to chromosomal mutations (Tagaino *et al*., 2019). These results are consistent with those of Yekta and Ansari (2019), who exhibited the mucilage addition at different concentration into yogurt reduced the acetaldehyde concentration. Acetaldehyde can vaporize or be transformed into ethanol by alcohol dehydrogenase that is secreted by culture starters of yogurt, and this one of the most important reasons for decreasing in acetaldehyde concentration during storage (Sahan *et al*., 2008).

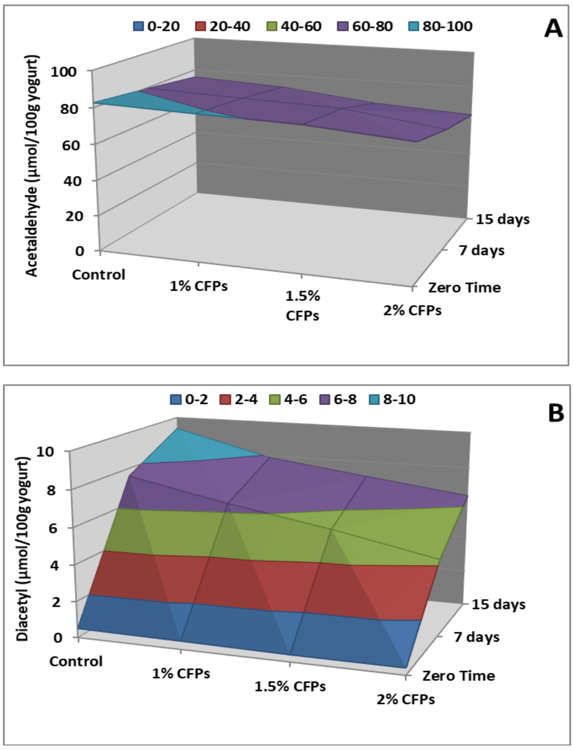

Figure 6. The impact of different CFP concentrations and storage duration on acetaldehyde (a) and diacetyl (b).

Diacetyl evaluation of yogurt

Figure 6b illustrates the variations in yogurt samples' volatile flavor components based on the diacetyl content. In contrast to acetaldehyde production, which rose considerably ($p>0.01$) throughout the storage duration at 4°C, diacetyl formation in yogurt samples was not affected by the length of storage. The diacetyl concentration in yogurt enriched with varying levels of CFPs (1%, 1.5%, and 2%) was lower than in the control across different storage periods (Figure 6b). These findings align with Kütt (2023), who found that in mixed cultures, the production of diacetyl increases with rising acidity, which is linked to the growth of the starter culture. A comparable trend was observed in yogurt fortified with inulin (Khalifa *et al*., 2011) and guar gum (Hassan *et al*., 2015). Regarding the current results, it may be stated that the CFPs as hydrocolloid can decrease the water activity and could cause negative effect for ability production of flavor compounds such as diacetyl and acetaldehyde by culture starter of yogurt.

CONCLUSION

The effect of adding different amounts of *Cassia fistula* polysaccharide (CFP) (1%, 1.5%, or 3% w/w) on the physicochemical properties of natural yogurt was examined. The study assessed physical stability (wheying-off and syneresis), texture parameters (hardness, adhesiveness, cohesiveness, springiness, gumminess, chewiness), and the formation of flavor compounds (acetaldehyde and diacetyl). Yogurt fortified with CFP exhibited significantly lower wheying-off and syneresis compared to the control, with the lowest values observed at a 2% CFP concentration. Hardness decreased as both the CFP concentration and storage time at 4°C increased. While adhesiveness was lower in CFP-enriched samples, it increased during storage. The addition of 2% CFP led to reductions in consistency, cohesiveness, and springiness, indicating a softer texture. Gumminess and chewiness were also lower in CFP-enriched samples, with the yogurt containing 2% CFP showing the lowest gumminess at 0.31 N. Acetaldehyde levels in CFP-enriched yogurt were lower than in the control and decreased over time, while diacetyl levels rose during storage but remained below those of the control.

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تأثير السكريات العديدة الخارجية المستخرجة من *Cassia fistula*على تكوين مركبات النكهة والخواص الريولوجية لليوغورت أثناء التخزين

محمد سمير درويش ، محمد شلبي جمعة و نوره علي حيدر

قسم الألبان – كلية الزراعة – جامعة المنصورة

الملخص

تم تحليل تأثير إضافة السكريات العديدة الخارجية المستخرجة من *Cassia fistula* (CFP) بتركيزات مختلفة (١ أو ١,٥ أو ٣٪) على الخصائص الفيزيائية والكيميائية للزبادي الطبيعي. تم التحقيق في دراسة الثبات الفيزيائي (التشريش بدون ضغط والتشريش تحت ضغط) وقياسات التركيب والقوام(الصلابة والالتصاق والتماسك والمرونة والصمغ والمضغ) وتكوين مركبات النكهة (الأسيتالديهيد وثنائي الأسيتيل). أظهر الزبادي المدعم بـ CFP انخفاضًا كبيرًا في معدلات التشريش بدون ضغط والتشريش تحت ضغط مقارنةً بالكنترول، حيث كانت اقل مستوياتهما في الزبادي المدعم بمعدل ٢٪ منCFP كما انخفضت الصلابة مع زيادة تركيز CFP ومع التقدم في وقت التخزين عند ٤ درجات مئوية. كانت القدرة على الالتصاق أقل أيضًا في العينات المدعمة بـ CFP ولكنها زادت أثناء التخزين. أدت إضافة ٢٪ من CFP إلى تقليل الالتصاق والتماسك والمرونة، مما يشير إلى قوام وتركيب أكثر نعومة. كانت الصمغية والمضغ أقل في العينات المدعمة بـCFP ، حيث أظهر الزبادي المدعم بـ ٢ CFP %أقل درجة من صمغية والتي وصلت إلى ما يقرب من ٠,٣١ نيوتن. كانت مستويات الأسيتالديهيد في الزبادي المدعم بـ CFP أقل من عينة المقارنة (الكنترول) وانخفضت بمرور الوقت، بينما زادت مستويات ثنائي الأسيتيل أثناء التخزين، ولكنها ظلت أقل من عينة المقارنة (الكنترول).

الكلمات الدالة: الالتصاق؛ التماسك؛ الصلابة؛ السكريات المتعددة؛ المرونة؛ التشريش