RESEARCH The impact of supplementing goats' milk with quinoa extract on some properties of yoghurt

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This study investigated the impact of supplementing goats' milk with quinoa extracts, in the range of 5, 10 and 15 g/100 g on the milk fermentation. The properties of yoghurt produced from this milk, which include viscosity, microstructure and sensory acceptability, were assessed. The supplementation of goats' milk with quinoa extracts, particularly permeate extract, reduced the fermentation time and enhanced the viability of lactic acid bacteria. Supplementation of yoghurt with increased levels of quinoa extracts increased the apparent viscosity and changed the yoghurt protein matrix. Panellists highly accepted the yoghurt that contained quinoa permeate extract.

Keywords Goats' milk, Yoghurt, Quinoa extract, Fermentation, Viscosity and microstructure, Sensory evaluation.

INTRODUCTION

According to the latest estimates of Food and Agriculture Organization (FAO), the world population of goats' is approximately 1 billion, of which Egypt breeds about 4.4 million (FAO 2017a). Goats' milk contributes approximately 2.3% of global milk production (FAO 2017b). In Egypt, the goats' milk production increased by 13.8% in 2017 as compared to 2007 (FAO 2017b).

Goats' milk has distinctive nutritional properties and certain therapeutic value (Park et al. 2007), which make it more attractive to some consumers as compared to milk from other species. However, processing of goats' milk into dairy products (i.e. yoghurt) is challenging (Delgado et al. 2017). Low viscosity, firmness and consistency and high susceptibility to syneresis (Joon et al. 2017) could adversely affect the quality and the acceptability of the product. Various attempts had been made to improve the textural and rheological properties of fermented goats' milk products. In this regard, the addition of traditional and functional ingredients (milk of other species and milk protein products) and the utilisation of new technological processes were applied (Delgado et al. 2017). Moreover, the addition of nondairy materials, such as soy protein, fruits, enzymes and polysaccharides, had been studied as well (Delgado et al. 2017). However, despite being promising, these approaches did not adequately meet the consumers' demand (Han et al. 2016).

In recent years, plant-derived food additives have received increasing attention, since studies have proven that these additives improve the technological, nutritional and health-promoting properties of the food product in which they are contained (Joung et al. 2016). Quinoa seed, Chenopodium quinoa, has gained much traction as an important food resource in the pharmaceutical and industrial sectors due to its superior nutritional value and ability to reduce the risk of various diseases (Maradini-Filho et al. 2017). In the food industry, quinoa is now commercially used to produce functional food products. Quinoa seed has a high content of carbohydrates, mainly starch, which makes up approximately 52–74 g/100 g of the seed dry matter (Maradini-Filho et al. 2017). Quinoa starch is characterised by its superior technological properties, such as higher amylograph viscosity, greater water binding capacity and swelling power, as

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compared to the starch of other seeds (Lorenz, 1990). Furthermore, quinoa starch grains are small in size and highly stable, making this starch applicable as substitute for chemically modified starches (Sharma et al. 2015). Recently, Codina *et al.* (2016) and Curti *et al.* (2017) successfully applied quinoa flour in cow's milk yoghurt, resulting in high viscosity and nutritional value. However, it negatively affected the texture and the overall acceptability when added at more than 1 g/100 mL in a product. As of to date, there are no published studies yet on using quinoa extracts in enhancing the textural and rheological properties of yoghurt, especially from goats' milk.

Membrane technology has procured considerable interest in the dairy industry in the recent years. Permeate, rich in lactose and minerals, is considered as a golden by-product produced from the modern membrane processes technology (Smithers 2008). Due to its high content of lactose, permeate is generally used as a sweet bulking, flavour enhancer and mild milk flavour provider (Stobaugh et al. 2016).

Taking all the aforementioned points into consideration, the present study aims to use milk permeate as a medium for quinoa extraction, which was then used for supplementing goats' milk in yoghurt production. The reason behind using permeate as a medium for extracting quinoa was to take advantage of permeate being rich in nutrients, a stimulator of the growth of starter culture and a flavour enhancer. Permeate and water were used to prepare quinoa extracts. Meanwhile, goats' milk was supplemented with water-soluble quinoa and permeate-soluble quinoa extracts at levels of 5, 10 and 15 g/100 g of milk. The influence of quinoa extracts addition on milk fermentation and viscosity, microstructural and sensory properties of yoghurts were investigated.

MATERIALS AND METHODS

Materials

Fresh goats' milk was obtained from Desert Research Centre, Ministry of Agriculture (Egypt). Quinoa seeds were purchased from the Egyptian Natural Oils Company (Cairo, Egypt). Commercially available lyophilised culture (Express 0.2, DVS) was supplied by Chr. Hansen Laboratories (Copenhagen, Denmark). Milk permeate, produced by ultrafiltration of fresh skimmed milk (UF Unit 9 SFEC, SFEC, 51 rue Ampere – 69780 Saint Pierre de Chandieu, France), was acquired from the Animal Production Research Institute, Agricultural Research Centre (Cairo, Egypt).

Preparation of quinoa extracts

The preparation of water and permeate extracts from quinoa is explained in the flow chart (Figure 1).

Preparation of yoghurt

Yoghurt was manufactured in triplicate, and each time goats' milk was divided into seven portions. The first

Figure 1 Preparation of water and permeate extracts of quinoa.

portion served as a control. Three portions of milk were supplemented with quinoa water extract at levels of 5, 10 and 15 g/100 g of milk. The other three portions were supplemented with quinoa permeate extract at the same levels. Yoghurts were manufactured as described by Tamime and Robinson (2007). Briefly, the goats' milk was pasteurised at 85 °C for 20 min and was immediately cooled to 42 °C before being inoculated with 2% (w/w) of starter culture, which was precultured in sterilised skimmed milk (0.02%, w/v) and incubated for 2 h at 42 °C before yoghurt production. Milk was incubated at 42 °C until the pH value was decreased to approximately 4.6. After which, yoghurt samples were transferred into a refrigerator at 4 °C and were stored for 1 day prior to analysis.

Methods of analyses

Proximate analysis (total solids, protein, ash (g/100 g)) of goats' milk, with or without quinoa extract, and titratable acidity (g lactic acid/100 mL) were determined in accordance with the AOAC (2005). The pH values were measured using a pH meter (Jenway 3505, Staffordshire, UK). The fat content (g/100 g) was estimated based on the method proposed by Folch et al. (1957). Starch $(g/100 g)$ was determined using the method suggested by Navale and Gupta (2015). All the analyses were carried out in triplicate.

Fresh yoghurt samples, after one day of cold storage, were evaluated for their microbiological quality, apparent viscosity, microstructure and sensory analysis as follows:

Microbiological analysis de Man, Rogosa and Sharpe (MRS) agar medium was used to enumerate viable cells of lactic acid bacteria in yoghurt samples, with the bacterial count (cfu/mL) being done in duplicate.

Apparent viscosity The apparent viscosity of yoghurt samples was measured in triplicate using Brookfield viscometer (model DV-II + Pro) (Brookfield Lab., Middleboro, MA, USA). The viscosities were measured at 8 ± 1 °C using the RV spindle (No. 4) and a rotation of 100 rpm.

Microstructure The microstructure of yoghurt samples was examined using transmission electron microscopy (TEM) (JEOL JEM-2100, USA). The method of Garcia-Risco et al. (2000) was used for the yoghurt samples preparation.

Sensory evaluation Yoghurt samples were served to seven trained panellists from the staff members of the Dairy Science Department, Faculty of Agriculture, Cairo University, Egypt. Each panellist was asked to evaluate yoghurt samples for sensory attributes. The quality rating scorecard was used for the evaluation of flavour (45 points), body and texture (40 points), acidity (10) and colour and appearance (5 points).

Statistical analysis

The data were analysed by a general linear model procedure (GLM) using SAS statistical analysis software package (SAS Procedure Guide 'Version 6.12 Ed.' SAS Institute Inc., Cary, 2004). The statistical analysis was performed using two-way analysis of variance (ANOVA). Means were compared by Duncan's test at the significance level of $P \le 0.05$. Pearson's correlation coefficient was used to calculate the correlation.

RESULTS AND DISCUSSION

Initial screening tests were performed in order to screen the effect of adding water-soluble quinoa and permeate-soluble quinoa extracts to goats' milk at different levels (5–45 g/ 100 g). Supplementation of goats' milk with quinoa extracts at different levels higher than 15 g/100 g resulted in a considerable increase in the viscosity of milk during the thermal treatment (85 \degree C/20 min), due to the high starch content at these supplementation levels. This viscosity increase made milk challengeable for being processed. Accordingly, the suggested addition levels were reduced to 5, 10 and 15 g/ 100 g.

Physicochemical properties of quinoa extracts and goats' milks

Tables 1 and 2 presented the physicochemical analysis of water and permeate extracts of quinoa, heated goats' milk (control) and milks supplemented with quinoa extracts.

The impact of supplementation with quinoa extracts on the mean value of the total solids content of goats' milk was significant ($P \le 0.05$), as shown in Table 2. The total solids increased, with a value falling in the range of 14.64 and $15.28 \text{ g}/100 \text{ g}$, compared to the control (12.81 g) 100 g). This increment is due to the presence of starch in quinoa extracts. During the heating of milk with quinoa extracts at 85 °C for 20 min, which is above starch gelatinisation temperature, starch granules absorb, bind water and swell (Jamilah et al. 2009). Because of the high water binding capacity of starch (Radi et al. 2009), it may retain some

Mean values (\pm standard deviation) within the same column with different superscript letters are significantly different at $P \le 0.05$.

Table 2 Physicochemical analysis of goats' milk and milk supplemented with quinoa extracts

Type of quinoa extract	supplementation level(g/100 g)	pH	Acidity $(g$ lactic acid/100 mL)	TS(g/100 g)	Fat(g/100 g)	Protein (g/100 g)	Ash (g/100 g)	Starch (g/100 g)
Control	θ		6.19 $0.15 \pm 0.01^{\circ}$	$12.81 + 0.30^{\circ}$	$4.06 + 0.36^a$	$3.11 \pm 0.0^{\circ}$	$0.69 + 0.02^{\circ}$	$0.00 + 0.0^{\text{t}}$
Water	5		6.25 0.14 ± 0.0^6	$15.09 + 0.20^{\circ}$		3.98 ± 0.19^a 3.04 \pm 0.01 ^a	$0.70 \pm 0.01^{\circ}$ $0.23 \pm 0.02^{\circ}$	
	10		6.17 0.14 ± 0.0^b	$15.25 \pm 0.32^{\circ}$		$3.7 + 0.01^a$ $2.99 + 0.27^a$	$0.81 \pm 0.03^{\circ}$ $0.43 \pm 0.02^{\circ}$	
	15		6.03 $0.19 + 0.01^a$	$15.15 + 0.13^{\circ}$		$3.48 + 0.21^a$ $2.97 + 0.10^a$	$0.72 + 0.01^{\circ}$ $0.52 + 0.03^{\circ}$	
Permeate	5		6.02 $0.19 + 0.01^a$	$15.28 + 0.16^a$	$3.85 \pm 0.22^{\circ}$ $3.03 \pm 0.0^{\circ}$		$0.79 + 0.05^{ab}$ $0.15 + 0.0^e$	
	10		6.19 0.18 ± 0.0^a	$14.64 + 0.26^b$			$3.44 \pm 0.33^{\circ}$ $3.02 \pm 0.11^{\circ}$ $0.73 \pm 0.04^{\circ}$ $0.37 \pm 0.05^{\circ}$	
	15		6.05 0.17 ± 0.01^{ab}	14.91 ± 0.11^{ab}	3.45 ± 0.24^a 2.95 ± 0.12^a 0.71 ± 0.01^c 0.49 ± 0.02^a			

Mean values (\pm standard deviation) within the same column with different superscript letters are significantly different at $P \le 0.05$.

bound water, preventing it from evaporating and consequently increasing the total solids of milk. On the other hand, there were no significant differences $(P> 0.05)$ in total solids between the treated milk samples in terms of the type and level of supplementation.

As for the starch, the type of quinoa extract and the level of supplementation showed a significant impact ($P \leq 0.05$) on the mean value of the starch content of treated milk samples (Table 2). Milk supplemented with quinoa water extracts had higher starch content than those with quinoa permeate extracts at all supplementation levels, with the exception of the level of 15 g/100 g. Furthermore, the starch content in milk increased by increasing the level of supplementation with quinoa extracts.

Fermentation process of goats' milk with quinoa extracts and viability of lactic acid bacteria in yoghurts

The effect of supplementing goats' milk with water or permeate extract of quinoa at levels of 5, 10 and 15 g/100 g on the milk fermentation is illustrated in Figure 2. The results show that the fermentation time for all milk samples was influenced by the type of quinoa extract and the level of supplementation. The supplementation of goats' milk with water or permeate extract of quinoa shortened the fermentation time (255 min for control vs 140 and 158 min for milk with permeate and water extract of quinoa, respectively, at a level of 15 g/100 g). Moreover, milks with quinoa permeate extract showed shorter fermentation time than those with quinoa water extract. In addition, by elevating the level of supplementation, the fermentation time was reduced. These results are inconsistent with the findings of Casarotti et al. (2014), who observed that supplementing fermented milk with quinoa flour at 0, 1, 2 or 3 $g/100 g$ had no effect on the fermentation time.

The reduction in fermentation time of milk samples containing quinoa extracts could be attributed to the more rapid changes in pH and titratable acidity as compared to the control. Furthermore, during the fermentation process, milk supplemented with quinoa permeate extract had higher rate of acid production and higher decline in pH than samples with quinoa water extract. The latter finding was clearly observed with the high level of supplementation. The mean values of titratable acidity (g lactic acid/100 mL) and pH values, as a result of supplementation, are presented in Figure 2(a,b), respectively. The rapid increase in the titratable acidity and the fast decrease in the pH value in supplemented samples could be related to the activity of lactic acid bacteria (LAB). Quinoa contains vitamins, minerals, amino acids, fermentable sugars and fibres (Gordillo-Bastidas et al. 2016), and all of which may have a stimulating effect on the activity of LAB. A similar behaviour was found by Codina et al. (2016), who reported that cow's milk yoghurt samples supplemented with quinoa flour exhibited a much higher decrease in the pH value and a much higher increase

Figure 2 Changes in titratable acidity (Mean \pm standard deviation) and pH values of goats' milk supplemented with quinoa extracts during fermentation.

in the acidity value as compared to the nonsupplemented samples throughout the time of fermentation. Moreover, the supplementation of goats' milk with quinoa permeate extracts may encourage the LAB growth due to the elevated concentration of lactose in milk with permeate extract.

The microbiological examination of the yoghurt samples was in parallel with these findings. As shown in Figure 3, in the absence of quinoa extracts, yoghurt contained the lowest number of viable LAB (77 \times 10⁶ cfu/mL), whereas, in the presence of quinoa extracts, particularly permeate extract, higher numbers of LAB were enumerated. Additionally, increasing levels of supplementation from 5 to 15 g/ 100 g increased the population of LAB by 14 and 21%, respectively, in yoghurts supplemented with quinoa water extract and by 18 and 29%, respectively, in yoghurts supplemented with permeate extract of quinoa. The results of Casarotti *et al.* (2014) revealed that adding up to 3 $g/100 g$ of quinoa flour to the fermented milk had a neutral impact

Figure 3 Viable lactic acid bacteria counts (Mean \pm standard deviation) of goats' milk yoghurts as impacted by supplementation with quinoa extracts.

on the activity of probiotic culture ABT-4 throughout the fermentation time and storage.

Apparent viscosity of goats' milk yoghurts with quinoa extracts

Viscosity is one of the important characteristics that determine the quality and consumer acceptability of yoghurt. Figure 4 demonstrates the effect of supplementing goats' milk with water or permeate extract of quinoa on the apparent viscosity (cP) of yoghurts. The supplementation of goats' milk with quinoa extracts increased ($P \leq 0.05$) the viscosity of yoghurts. The lowest apparent viscosity was recorded for the control yoghurt sample. The size of fat globules and casein micelles, as well the relative proportions of casein fractions of goats' milk, could be responsible for the low viscosity of the control yoghurt (Park et al. 2007). Goats' milk contains a low percentage of α s-casein and a high content of β -casein. Moreover, the weak structure and texture

Figure 4 Apparent viscosity (Mean \pm standard deviation) of goats' milk yoghurts as impacted by supplementation with quinoa extracts.

of goats' milk yoghurt probably attributed to the high content of nonprotein nitrogen and the low content of casein nitrogen (Guo 2003).

Increasing the apparent viscosity of treated yoghurts can be explained by the presence of starch in quinoa extracts. Heating starch above its gelatinisation temperature in the presence of water makes granules absorb and bind water, swell and disrupt their structure, and thus change their rheological properties (Jamilah et al. 2009). According to Jancurová et al. (2009), the gelatinisation temperature of quinoa starch ranges from 57 to 64 $^{\circ}$ C. In the current study, heating both milk and quinoa extract that contain starch at 85 °C for 20 min prior to the starter inoculation was adequate for starch gelatinisation to occur in milk. In this mechanism, the swelling of starch granules and leaching of amylose (primarily) and amylopectin in the continuous phase (Oh et al. 2007; Jamilah et al. 2009) might have increased the viscosity of yoghurts. Likewise, Williams et al. (2003) observed an increase in viscosity of stirred yoghurt when a modified starch was added to milk prior to heating. The increase in viscosity could also be attributed to the modification of the gel structure by forming casein– starch complex system. Jamilah et al. (2009) demonstrated that the interaction between starch and protein in food systems augmented the gel strength, due to an increase in the density of protein matrix and formation of elastic starch globules. Other quinoa carbohydrates, such as fibres and other polysaccharides, may also be responsible for enhancing the apparent viscosity. Previously, Codina et al. (2016) attributed the increment in the viscosity of cow's milk yoghurt fortified with up to 1 g/100 mL of quinoa flour to its high-fibre content. The high total solids content in the milks supplemented with quinoa extracts could be another reason for the higher viscosity of yoghurts than the control.

The statistical correlation between the apparent viscosity of yoghurts and the major components of milk supplemented with quinoa extracts was determined. The results revealed that the correlation between the values of apparent viscosity and the starch content was highly significant (Pearson's correlation coefficient, $r = 0.96$). Meanwhile, the relationship between the apparent viscosity and total solids was significant ($r = 0.48$). In other words, the influence of starch content on the apparent viscosity was greater than the impact of total solids on the same parameter. On the other hand, the content of total protein and fat had no effect on the apparent viscosity of yoghurt samples.

The type of quinoa extract had a significant ($P \le 0.05$) effect on the apparent viscosity of yoghurts. As evident in Figure 4, the viscosity of yoghurt samples prepared with quinoa water extract was higher than those prepared by quinoa permeate extract at all levels of supplementation, except when the level was 15 g/100 g. This finding could be due to the higher content of starch in milk supplemented with quinoa water extract than those with quinoa permeate

Figure 5 (a) Transmission electron micrographs of goats' milk yoghurts with and without quinoa extracts. (b) Starch/total solids ratio. The bar corresponds to 1 µm. F: fat globule, S: starch.

		Type of quinoa extract							
		Water $(g/100 g)$			Permeate $(g/100 g)$				
Sensory attribute	Control	.5	10 ²	1.5	5	10	15		
Flavour (45)	$32.3 \pm 2.1^{\circ}$	$39.3 + 2.5^{\rm b}$	$40.3 + 1.5^{ab}$	$39.3 \pm 3.8^{\rm b}$	$42.3 + 2.1^{ab}$	$43.7 \pm 0.6^{\circ}$	$44.0 + 0.0^a$		
Body $&$ Texture (40)	$18.3 \pm 5.8^{\circ}$	$29.0 \pm 3.5^{\rm b}$	33.0 ± 0.0^{ab}	$35.0 \pm 0.0^{\circ}$	$33.3 + 2.1^{ab}$	$34.7 + 1.5^{\circ}$	$36.0 \pm 1.7^{\rm a}$		
Acidity (10)	$6.3 \pm 1.2^{\circ}$	$7.7 \pm 0.6^{\rm b}$	8.0 ± 0.0^{ab}	$7.7 \pm 0.6^{\rm b}$	$9.0 \pm 0.0^{\circ}$	$8.7 \pm 0.6^{\rm ab}$	8.7 ± 0.6^{ab}		
Colour & Appearance (5)	$3.3 \pm 0.6^{\rm b}$	$4.3 \pm 0.6^{\circ}$	$4.3 \pm 0.6^{\circ}$	$4.3 \pm 0.6^{\circ}$	$4.8 \pm 0.3^{\rm a}$	$4.7 \pm 0.3^{\circ}$	$4.8 \pm 0.3^{\rm a}$		
Total (100)	$60.3 \pm 5.9^{\circ}$	$80.3 \pm 6.7^{\rm b}$	$85.7 + 2.1^{ab}$	86.3 ± 4.0^{ab}	89.5 ± 0.9^{ab}	$91.7 + 1.6^a$	$93.5 + 1.8^{\rm a}$		

Table 3 Sensory evaluation of goats' milk yoghurts as impacted by supplementation with quinoa extracts

Mean values (\pm standard deviation) within the same row having different superscript letters are significantly different at $P \le 0.05$.

extract. Also, the increase in the supplementation level considerably improved the apparent viscosity of yoghurts. At the level of 15 g/100 g, the apparent viscosity of yoghurt samples dramatically increased by approximately 800% for both types of supplementation as compared to the control samples.

Microstructure of goats' milk yoghurts with quinoa extracts

In this study, TEM was used for visualising the changes in the aggregation of casein micelles in produced yoghurts, as a result of quinoa extracts supplementation after 1 day of storage at 4 °C. For all yoghurt samples, TEM examination showed chains of aggregated casein particles and fat globules of different arrangements and structures. Kalab et al. (1983) reported that many factors, especially thickening agents, affect the way in which casein micelles may interact.

The micrographs of control yoghurt sample (Figure 5a) showed that fat globules of different sizes were imbedded in the structure and that the yoghurt matrix consisted mainly of casein micelles arranged in dense longitudinal polymers. The protein matrix also contained void spaces with aqueous phase retained.

The supplementation of goats' milk with both quinoa extracts at levels of 5, 10 or 15 g/100 g caused changes in the microstructure of yoghurts compared to the control samples. These changes were associated with an increase in starch/total solids ratio (Figure 5b). Also, TEM micrographs (Figure 5a) revealed that yoghurts which contained quinoa extracts possessed more compacted microstructure than the control, in which the starch appeared to be surrounded by aggregated protein particles and occupied the void space with various dimensions within the casein particle network. This result was in line with the findings of Haque and Aryana (2002). In addition, yoghurt made with quinoa extracts at levels of 5 and 10 g/100 g had a matrix in which casein micelles tended to aggregate and connect in the form of double linear clusters. At the highest level of addition $(15 \text{ g}/100 \text{ g})$, the starch/total solids ratio represents the highest value, which filled the void spaces between casein

micelles and thus led casein micelles to aggregate around starch particles.

Sensory evaluation of yoghurts with quinoa extracts

Table 3 presents the effect of supplementation of goats' milk with water or permeate extracts of quinoa on flavour, body and texture, acidity as well as colour and appearance of yoghurts. Data show that supplementing goats' milk with quinoa extracts enhanced the sensory attributes of yoghurts. The control sample had the lowest score for flavour. This could be owing to the goaty flavour, which is highly related to the volatile fatty acids, caproic, caprylic and capric (Mele et al., 2008). This flavour almost diminished by using quinoa extracts. This result is probably due to the enhancement in the growth of starter culture, which increases the production of lactic acid and other flavour compounds, consequently masking the goaty flavour. Also, yoghurts with quinoa permeate extracts showed higher acceptability for flavour than those with quinoa water extracts at all supplementation level. As shown in Table 3, treated yoghurts had higher acidity and were more acceptable than control samples.

Yoghurts with quinoa extracts scored the highest for body and texture, whereas the control samples scored the lowest. This finding suggests that the supplementation had a positive impact on this parameter, which was remarkable with the high levels of supplementation. In addition, supplementing goats' milk with quinoa extracts significantly enhanced the colour and appearance of treated yoghurt samples as compared to the control samples. In general, yoghurt samples with quinoa permeate extract received the highest overall acceptability at all levels of supplementation, as indicated by the assessors.

CONCLUSIONS

Supplementation of goats' milk with water or permeate extract of quinoa, particularly at levels of 10 and 15 g/ 100 g, enhanced the quality of the final products in terms of apparent viscosity, microstructure and organoleptic acceptability. Quinoa extract containing starch can be used as a

texture enhancer to overcome the weak texture of goats' milk yoghurt. Furthermore, the supplementation with quinoa permeate extract enhanced the viability of lactic acid bacteria and led to a reduction in the fermentation time, which is considered an important criterion in yoghurt production.

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