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Effect of Carrot Pulp to Improve Textural and Viable Counts Cell of Yoghurt

Reda M.Y. Morsi [1] and Wahid Ahmed Ragab[2]

[1]Department of Special Food and Nutrition, Food Technology Research Institute, Agricultural Research Center (ARC), Giza, Egypt.
[2]Department of Dairy Technology Research, Food Technology Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

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ABSTRACT

Yoghurt is one of the most popular fermented dairy products which has a wide acceptance worldwide and is considered as a healthy food due to its high digestibility and bioavailability of nutrients. Nowadays consumers move towards functional foods with specific health benefits. The present study aims at shedding light onto the carrot juice as an antioxidant source as well as improving the flavor and functional properties of yogurt after short-term cold storage. Carrot juice were added at concentration of 2, 4 and 6% to enhanced products textural, WHC, and TPA either after 1, 7 and 14 days of cold storage. Carrot juice stimulated the growth of the lactic acid bacteria, whose counts dramatically increased compared with plain yoghurt at 1, 7 and 14 days of storage period. Finally, use of carrot juice in preparation of yoghurt from cows' milk resulted a product with good quality of texture and viable account cell plus same shelf life. In addition, the prepared yoghurt samples were highly accepted of pH, and acidity with all ratios 2,4 and 6 %. The hardness, adhesiveness, gumminess, chewiness values of industrial yogurt samples were higher, while springiness, and cohesiveness and resilience values were lower than plain yogurt samples. Further research could be conducted on the components of the active compounds found in carrot yoghurt yogurt and more research is needed to investigate the therapeutic effects of carrot yoghurt.

Keywords: Carrot pulp, yoghurt, Texture profile analysis (TPA), Radical scavenging activity (%RSA), Microbiological characteristics, Sensory evaluation.

1. Introduction

Yoghurt is one of the most popular fermented dairy products which has a wide acceptance worldwide and is considered as a healthy food due to its high digestibility and bioavailability of nutrients (Weerathilake *et al.,* 2014). It can be recommended to the people with lactose intolerance, gastrointestinal disorders, and aids in immune function and weight control (Mckinle 2005).

Nowadays consumers move towards functional foods with specific health benefits, blending yoghurt with carrot powder will provide consumers with a nutritionally balanced food. In efforts to offer variety and competition in the market, new research is currently in progress on the use of carrot juice in yoghurt industry (Simova *et al.,* 2004). The results of (Salwa *et al.,* 2004) highlighted the possibility of processing yoghurt with 15% carrot juice.

Texture is one of the main characteristics that define the quality of yogurt and affect its appearance, mouth feel and overall acceptability (Yoon and McCarthy 2002). The most frequent defects related to yogurt texture, which may lead to consumer rejection, are apparent viscosity variations and the occurrence of syneresis (Keogh and O'Kennedy 1998).

Carrot *(Daucus carota* L.) is one of the popular root vegetables grown throughout the world and is one of the more commonly used vegetables for human nutrition. It is rich in beta carotene, ascorbic acid, tocopherol and classified as vitaminized food (Hashimoto and Nagayama, 2004). The intake of carrot as potent antioxidants, appear to be associated with better health. It is not only

Corresponding Author: Reda M.Y. Morsi, Department of Special Food & Nutrition, Food Technology Research Institute.ARC, Giza, Egypt.

preventing vitamin A deficiency but also cancer and other diet related human diseases (Salwa *et al.,* 2004**)**. Carrots are good source of iron and vitamins A and C but lack in protein and fat. Yoghurt is rich in protein and fat but is deficient in iron and vitamin C, therefore blending yoghurt with carrot will produce a nutritionally rich food (Raum, 2003).

Khanum *et al.* (2000) reported carrots are high in dietary fibers and these fibers play an important role in human health and the diets rich in dietary fibers are associated with the prevention, reduction and treatment of some diseases. Nawirska and Kwasniewska (2005) have reported the composition of dietary fiber constituents in the fresh carrot on dry weight basis as pectin (7.41%), hemi-cellulose (9.14%), cellulose (80.94%) and lignin (2.48%).

On other hand, phenolics could play an important role in antioxidant properties in carrots. Phenolics in carrots are present throughout the roots but are highly concentrated in the periderm tissue (Mercier *et al.,* 1994). Two major classes of phenolics are hydroxycinnamic acids and para-hydroxybenzoic acids (Babic *et al.,* 1993).

Blending of yoghurt with carrot juice produces a nutritionally rich food (Raum 2003). Excellent quality carrot-yoghurt could be prepared by blending milk in different proportions having 5–20% carrot juice before fermentation (Salwa *et al.,* 2004).

Technological and milk compositional parameters, like total solids content, temperature, food ingredient fibers (carrot juice, oligofructose and others), exopolysaccharides and synergistic interactions between microorganisms, have been reported to influence the structural characteristics of fermented products. Since all these factors modulate the growth of lactic acid bacteria (LABs), they are in fact decisive to improve the quality of dairy products and consequently their sensory characteristics and physical properties such as firmness and viscosity (Donkor *et al.,* 2007).

LABs are well-known probiotics. Conceptually, probiotics are live microorganisms which confer beneficial effects for host health when they are consumed in appropriate quantities (FAO/WHO, 2002). However, to produce them, probiotics should be present in the product in viable counts during their whole shelf-life. It has been recommended that the minimum dose able to assure therapeutic effects should be in the range 7- 9 log CFU/mL (Kailasapathy and Rybka, 1997).

Several LABs, such as *Streptococcus thermophilus*, *lactobacilli and bifidobacteria*, are able to direct some part of the sugar pool toward biosynthesis of exopolysaccharides (EPSs). As a result, EPSs can improve technological characteristics of fermented dairy products, like stability and texture, and may also offer protection to cells against phage attack, desiccation and osmotic stress (Ruas-Madiedo *et al.,* 2007), thus behaving as prebiotic.

On the basis of this background, the effect of carrot juice and the symbiotic interactions between probiotics deserve to be explored for the production of new dairy functional foods with improved quality. To this purpose, the present study aims at shedding light onto the carrot juice capability to improve the firmness, syneresis and viable cell counts after short-term cold storage, of caw's milk fermented by *Lactobacillus bulgaricus* and *S. thermophilus*. So, this research was designed to study the stability and phytochemicals of carrot juice incorporated yoghurt and find the best combination of carrot juice and fermentation duration. In addition to utilizing the carrot juice (in context to Egypt) as a potential source of bioactive components in yoghurt.

2. Materials and Methods

2.1. Raw material

The carrot tubers obtained from local market and washed thoroughly with water, peeled and cut longitudinally into halves. These halves were steam blanched for five minutes to inactivate pectinase and peroxidase enzymes, in addition to tenderize the carrot tissues, continue to cook until soft and was mashed.

Yoghurt culture containing *Streptococcus salivarius subsp. thermophilus* and *Lactobacillus delbruekii subsp. Bulgaricus* Were obtained from the Microbiological Resources Center (MIRCEN), Faculty of Agric., Ain Shams Univ., Egypt. Raw cow's milk was obtained from the desert development center dairy farm (Bader city, EL-Beheira, Egypt), in sterilized containers with sieves and transferred to the laboratory under aseptic conditions.

2.2. Milk and inoculum preparations

Cow's milk mixed with 2%, 4% and 6 % of carrot pulp and stirred. Both were thermally treated at 90 °C for 15 min in a water bath, then transferred to sterile flasks, cooled in ice bath to 40°C, distributed into 250-mL sterile Schott flasks inside a laminar flow chamber. Culture was weighted in sufficient amounts (130 mg of freeze-dried culture in 50 mL of sterilized skim milk) to get initial counts of 10^6 CFU/mL, and activated at 40 °C for 15 min before use.

2.3. Fermentations

Mixture of milk and carrots were inoculated by 2% of starter culture, samples were transferred to a water bath, and batch fermentations were performed in triplicate at 40 °C up to pH 4.5, which were selected as the conditions to stop the fermentation. Flow diagram of yoghurt processing according study, are shown in (Fig - 1).

Cow's milk supplemented with carrot pulp (puree)

↓

Heated to (90°C for 15 min)

↓

Cooling to 40 °C

↓

Inoculation with starter culture of **2**%

↓

Mixing

↓

Incubation at 40°C for 3-4 hr, until coagulation.

↓

Cooling and quality evaluation.

Fig. 1: Flow diagram for the preparation of yoghurt with carrot pulp.

Yoghurt treatments with 2%, 4% and 6% carrot pulp were transferred to cool storage at 5°C, and kept for 14 days and analyzed for their physicochemical, microbiological, rheological and sensory properties at zero-time and after 7 and 14 days of storage.

2.4. Chemicals analyses

4-1- Carrot pulp and resultant yoghurt from all treatments were analyzed for total solids and titratable acidity (Association of Official Analytical Chemists, AOAC, 2010).The pH of the samples was measured by using a digital pH meter.

4-2-Total nitrogen percentages were determined by semi-micro Kjeldhel method as described in the AOAC (2010).

4-3- Acetaldehyde and Di-acetyl contents in all yoghurt treatments were determined as described by AOAC (2010). Whereas, acetaldehyde reacts with semi-carbazide to form semi-carbazone which has absorption value at 224 nm wave length meanwhile Di-acetyl has an absorption value at 270 nm.

4-4- Total volatile fatty acids (TVFA) of all yoghurt treatments were estimated according to AOAC (2010.

2.5. Radical scavenging capacity of yogurt (DPPH):

In 375 µL ethanol (99%) and 125 µL DPPH solution (**1,1-Diphenyl-2-picrylhydrazyl)** sample volumes of 500 µL were supplemented as a free radical source at different concentrations. (25 µg/mL, 50 µg/mL, 75 µg/mL, 100 µg/mL, and 125 µg/mL). At room temperature (25°C) in the dark, the mixture was shacked and incubated for 60 min. A spectrophotometer was used to measure the radical scavenging capacity and control absorbance at 517 nm. DPPH has a 517 nm absorption band, which is eliminated when anti-radical compounds are reduced. The lower absorption level indicates that the reaction mixture likely possesses increased radical scavenging activity. The DPPH radical scavenging capacity is calculated according to Zhang D and Hamauzee Y (2004), as follows:

$$\%RSA = (A\ control - A\ sample) / A\ control \times 100$$

2.6. Rheological measurements by Texture profile analysis (TPA)

Texture profile analysis test of samples Which shape was 2*2*2 cm cylindrical) was done in Food Technology Research Institute using a Universal Test Machine (TMS-pro) Food Technology Corporation, Sterling, Virginia, USA)equipped with 1000N (250lbf)load cell and connected to a computer programmed with texture pro with TM texture analysis software (program, DEV TPA with holding time between cycles two second). A flat rod probe (49.95 mm in diameter) was used to uniaxially compress the samples with the following parameters condition to 30 % of their original height. The TPA test set condition was adjusted to a test speed 50 mm / sec; trigger force IN, deformation 40 % and holding 2 sec between cycles). Each sample was subjected to two subsequent cycles (bites) of compression – decompression .Data were collected on computer and the texture profile parameters were calculated from DEV TPA texture analyzer and computer interference. Calculation described by (Bourne,2002) was used to obtain the following texture profile parameters (Adhesiveness, Cohesiveness,Springiness, Hardness, and Gumminess).Whereas, the parameters simulating included hardness (the peak force during the first compression cycle), adhesiveness(the negative force area of the first compression cycle), cohesiveness (the ratio of the positive force area during the second compression cycle to that during the first compression cycle) and springiness (the length to which the sample recovers in height during the time that elapses between the end of first compression cycle start of the second compression cycle).

2.6. Microbial analysis

For evaluation of microbial viable counts of yoghurt samples, appropriate dilutions of the respective sample in 1 ml aliquots were surface plated on pre-poured plates of Nutrient agar for total plate counts, MRS agar for lactic acid bacteria, Mac Conkey agar for the enumeration of Presumptive coli form test and Potato dextrose agar for moulds and yeasts. While, the Nutrient agar plates and Mac Conkey agar plates were incubated for 24-48 hrs, while, the PDA plates were incubated for 72 hrs at 25°C. Characteristics colonies appearing on the respective selective agar media were counted and multiplied by the dilution factor and expressed as colony forming units per milliliter (cfu/ ml). All microbiological analyses were carried out on different samples of yoghurt stored at 5 ºC for storage times,1 day, 7 days,14 days, according to (Sivakumar, and Kalaiarasu, 2010).

Colony forming units (CFU) were enumerated in plates containing 30–300 colonies, and cell concentration was expressed as log CFU/mL;(Viable cells were calculated as follows:

% Viability of total lactic acid bacteria = (CFU at n week(s) of storage/initial CFU) × 100)

2.7. Sensory evaluation

Ten staff members, aged between 30 to 60 years), performed sensory evaluation on fresh carrot yoghurt samples at Dept of Dairy Milk, Food Technology Research Institute, ARC, Giza, Egypt. The criterion for selection depended on their experience and background related to yoghurt products. The samples were allowed to rest at room temperature (25ºC), 10 min before evaluation. Panelists were instructed to evaluate the carrot yoghurt with respect to their degree of acceptance, 20 g portions of each yoghurt sample and used a quality rating score card for evaluation as follows; flavor as smell and taste (60 points), body and texture (30 points), and appearance (10 points). The sensory evaluation procedure was modified from the method described by Nelson and Trout (1981). The average was calculated.

2.8. Color analysis of carrot pulp and carrot yoghurt:

For color, a Hunter lab (Colorflex, VA, Model 45/0, USA) was used to measure the L, a*, and b* color parameters at 25°C.

2.9. Statistical analyses

Sensory evaluation were analyzed by the analysis of variance (ANOVA) according to SAS (1982). To determine whether there were significant differences between means for each variable, least significant difference (LSD) test was used.

3. Results and Discussion

As presented in Table 1 moisture content of carrot pulp represents its major component (87.78%), while total soluble solids, acidity, total sugars and total carotenoids were 11%, 2.55%, 7.0%, and 10.9 mg/100g respectively, while in (Table 2) with regard to the characteristic aroma compounds of yogurt; that is, diacetyl and acetaldehyde, it is important that the addition of carrot pulp does not negatively affect the typical flavor of the product. The results obtained in this study are promising in that, throughout the storage period evaluated, the yogurts with carrot pulp were found to produce greater amounts ($P < 0.05$) of these 2 compounds compared with the control yogurt.

As for diacetyl, the values determined in the experimental yogurts varied from 14.62 to 15.11 mg/L of 2,4% carrot yoghurt, comparison with control which was reduced to 13.64 mg/mL after 14 days.

Greater production of acetaldehyde ($P < 0.05$) was also observed, which is extremely favorable because this compound is mainly responsible for the typical aroma of yogurt (Cheng, 2010). Maximum production (68.5 µg/mL) was observed for 6% carrot pulp after 14 days of refrigerated storage, comparison with control. The results are coherent, because overall, probiotic bacteria do not produce aroma compounds markedly and, in general, probiotic yogurts and fermented milks are low in flavor due to the low activity of the enzyme threonine aldolase, which catalyzes the formation of acetaldehyde from the substrate, threonine (Gardini *et al.,* 1996).

Table 1: Chemical composition of Carrot pulp fresh on wet weight basis.

Moisture %	Protein %	Crude oil %	Crude fiber %	Ash %	Carbohydrate %	pH	T. carotenoids (mg/100g)	Acidity %
87.78	0.75	0.25	2.5	1.50	7.0	5.8	10.9	2.55

Table 2: Physicochemical properties and coagulation time of yoghurt.

Ingredients	Yoghurt with carrot pulp			
	Control	**2%**	**4%**	**6%**
Fat	3.37	3.34	3.37	3.33
Total solids%	13.4	12.80	12.3	11.8
Total nitrogen%	0.64	0.62	0.61	0.57
Fat%	3.71	3.54	3.34	3.21
TVFA**	0.26	0.23	0.21	0.18
Acetaldehyde (µg/mL)	65.5	66.3	67.2	68.5
Diacetyl (µg/mL)	13.64	14.62	15.11	15.42
Lactic acid (mg/mL)	4.78	4.88	4.9	5.2
Coagulation time (min)	160	160	165	170

TVFA= Total volatile fatty acids.

In this context, adequate production of acetaldehyde and diacetyl, which are the compounds related to the typical flavor of the yogurt (Cheng, 2010), is essential.

On the other hand, Baranowska (2006) reported that levels of lactose and glucose present in milk did not influence the production of diacetyl and acetaldehyde in yogurt, but did influence supplementation of the yogurt with the precursors of aroma compounds. A recent study (Pinto *et al.,* 2009) confirmed this finding when investigating the behaviors of diacetyl and acetaldehyde in yogurts processed with single and joint cultures throughout shelf life. The authors showed that the amount of each compound produced depended on the cultures used and the interactions between them. In general, the production of diacetyl was greater with yogurts fermented by *Strep. thermophilus* on its own, also the production of acetaldehyde was greater in yogurts fermented with *Lb. bulgaricus*. Ott *et al.* (2000) report that the difference in production of flavor compounds in yogurt may be related to the intensity of the acidity of the products.

Recently, Cachon *et al.* (2011) reported on the influence of the oxidoreduction potential value on the production of aroma compounds, as it can modify the metabolic pathways of the yogurt bacteria. Oxidative conditions contribute to the stability of the acetaldehyde during the yogurt storage, whereas reducing conditions provide the opposite effect.

Our approach is relevant because we observed no negative effect of carrot pulp supplementation on production of aroma compounds in yogurt. This is crucial for its acceptance by the consumers as probiotic and conventional yogurts should be similarity for consumers. In addition, the typical yogurt aroma compounds, acetaldehyde and diacetyl, were found to be produced in adequate amounts, together with the formation of moderate amounts of organic acids, particularly lactic acid.

Texture profile analysis of carrot yoghurt products are given in Table (3). Comparing the hardness of the control sample and 2%, 4%, 6% carrot pulp, revealed that hardness is inversely proportional with chemical composition content. The same pattern was obtained when comparing amongst each control yoghurt, where softness significantly elevated directly proportional with increasing of carrot pulp concentration. Data of adhesiveness and gumminess values revealed significant descend following the same trend of hardness. On contrary, springiness showed significant differentiation accompanied with the increase of carrot pulp concentration and storage period. Back to springiness definition (originally called elasticity), that it is the rate at that a deformed sample returns to original size and shape after the force is removed (Trinh & Glasgow, 2012), that could be interpreted when referred to the fibrous nature of supplemented carrot pulp

On the other hand, cohesiveness (strength of internal bonds in the sample) did not significantly affect with carrot pulp supplementation. Overall TPA result indicted that yoghurt supplementation with carrot pulp dispersion, positively affected its texture to be softer and smoother with strong internal bonds that increased its resilience, which was connected to organoleptic evaluation when carrot yoghurt described as "soft". These properties could be connected to the relatively fiber and protein, and mineral content of carrot pulp.

Table 3: Texture profile analysis of carrot yoghurt products

Sample / Storage period	Hardness (g)	Adhesiveness (g.mm)	Cohesiveness	Springiness (mm)(H*Co)	Gumminess (N)(H*Co)
Control (first day)	108a	72.13a	0.37a	5.86b	39.96a
2%	40b	45.27b	0.44a	7.44a	17.6b
4%	35c	28.78c	0.44a	6.55c	15.05c
6%	28d	27.88c	0.45a	4.88d	12.6d
Control (7 days)	101a	48.66a	0.38a	7.32a	37.37a
2%	64b	42.33b	0.45a	6.89a	22.4b
4%	51c	41.22c	0.46a	5.88b	17.34c
6%	49d	36.90d	0.46a	5.90b	17.15d
Control (14 days)	73a	29.65c	0.40a	5.88b	29.93a
2%	61b	26.93d	0.46a	5.44a	28.06a
4%	50c	24.04a	0.47a	6.42a	21.5b
6%	48d	21.46b	0.49a	6.33a	20.16b

S. thermophilus, *Lactobacillus spp*. are well known to influence the fermented milk textural properties, and the effects of these microorganisms and low temperature storage on firmness or coheviness have already been studied (Donkor *et al.,* 2007; Tamime, 2005). However, tests carried out in this work by Texture Analyzer on milk fermented by pure culture demonstrated that only St induced sufficient firmness or Cohesiveness. This result is consistent with the well-known fact that St is the microorganism responsible for yoghurt firmness and explains why it is used in industrial preparations in much higher enumeration than the other lactic bacteria.

As the likely result of interactions between microorganisms and carrot pulp. Such an interaction, probably characterized by a synergistic effect, could be explained with the discovery that *S. thermophilus* produces little amounts of formic acid and CO_2 (Louaileche *et al.,* 1993**)** that can stimulate the growth of lactobacilli, while *Lactobacillus* spp. are able to release peptides by a serine protease which stimulates the growth of *S. thermophilus* (Nakada *et al.,* 1996). It is well known in fact that higher microbial growth is one of the causes of a firmness increase in yoghurts (Donkor *et al.,* 2007 and Tamime, 2005).

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Consistently with previous observations in similar system (Damin *et al.,* 2008), St–Lr exhibited statistically higher ($p < 0.05$) firmness or Cohesiveness (0.38–0.55 N) than the other co-cultures (0.33–0.47 N), as a consequence of the slow fermentation ability of *L. rhamnosus*.

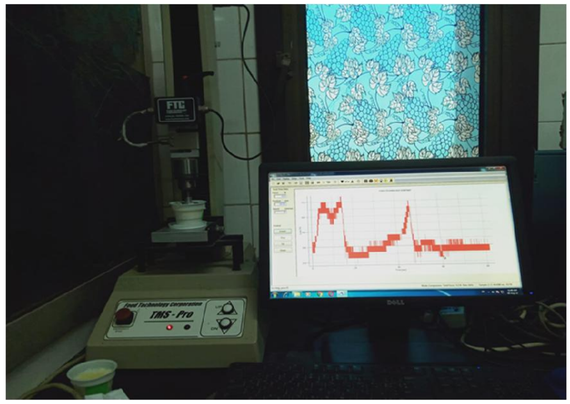

Fig. 2: Instrument of TPA.

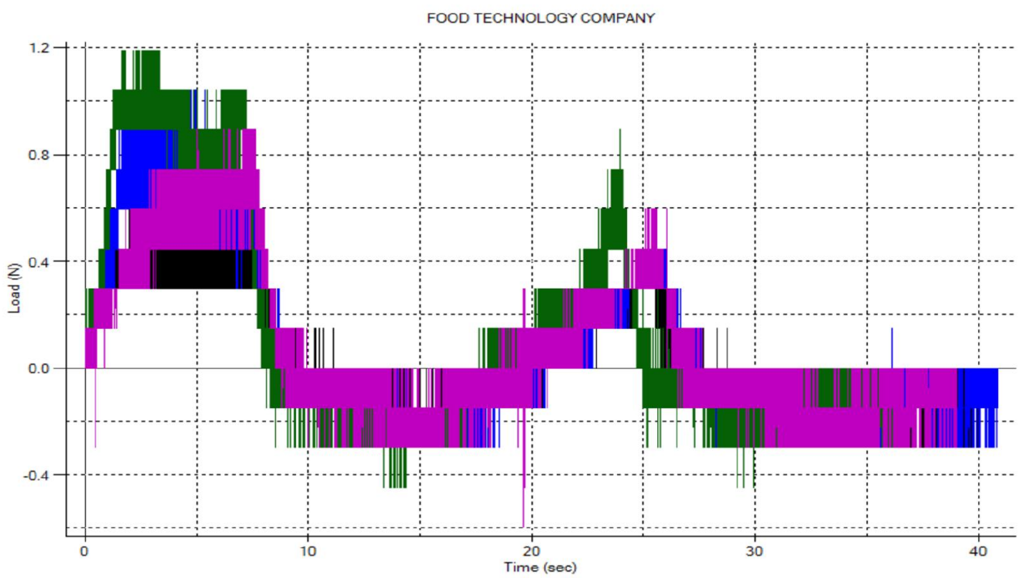

Fig. 3a: Shows a graph of yoghurt samples after first day of storage

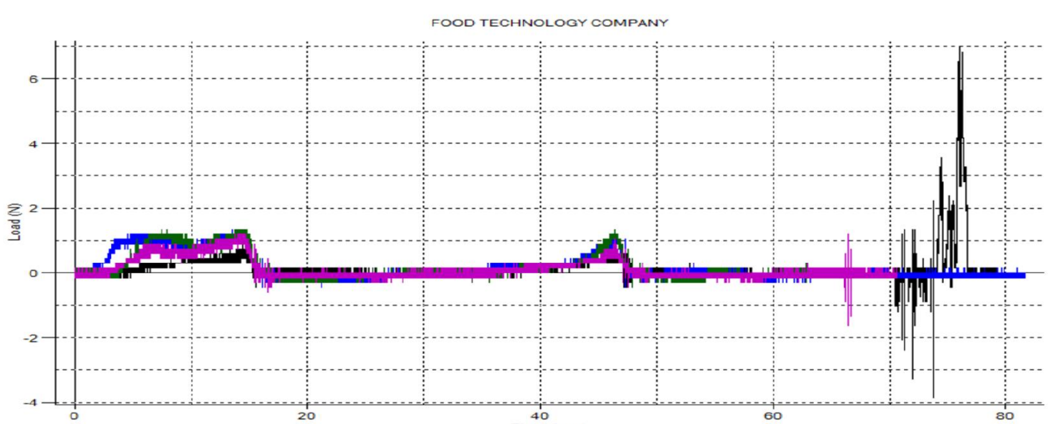

Fig. 3b: Shows a graphs of yoghurt samples after 7 days of storage

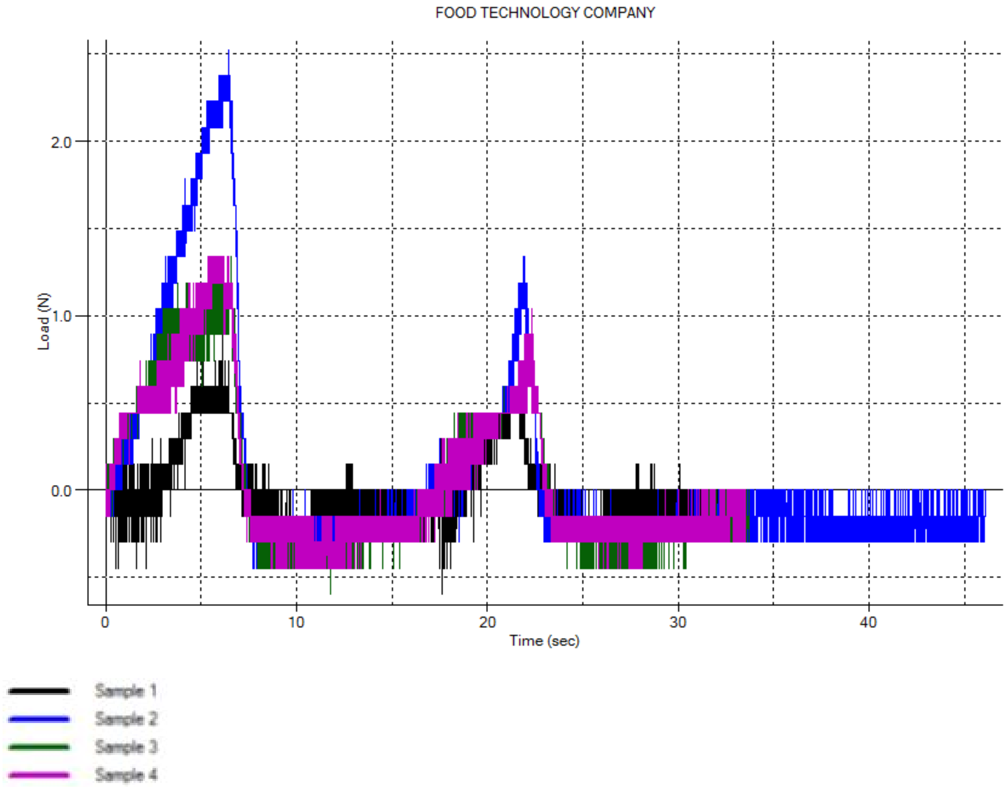

Fig. 3c: Shows a graph of yoghurt samples after 14 days of storage

The results also demonstrate that the firmness or cohesiveness of the carrot yoghurt was enhanced significantly not only by carrot pulp addition as a prebiotic, but also by an increase in the time of cold storage. As far as the effect of storage time on firmness is concerned, such an increase was consistent with the effect of time on the moisture content during storage (Andreatta *et al.,* 2009).

On the other hand, the enforcing effect of prebiotic has been justified in the literature by a number of different causes. First of all, the direct dependence of firmness Cohesiveness on the total solid content (Kristo *et al.,* 2003,Tamime, 2005), which increased in this study from 0.132 to 0.156 g/g as the result of inulin addition. According to Robinson (1999), inulin would raise the viscosity as a consequence of the higher total solid content, hence increasing the firmness or cohesiveness .The increase in viscosity due to the addition of fiber has also been attributed to interactions between oligo- or poly-saccharides, and dairy proteins (Sodini *et al.,* 2002).

One additional hypothesis to explain a prebiotic can stimulate the metabolism of LABs, as the likely result of the increased level of fructose released from its partial hydrolysis, which was metabolized as an additional carbon and energy source (Tamime, 2005). According to Ruas-Madiedo *et al.,* (2007) the firmness increase may be due to the release of exopolysaccharides (EPSs) from glucose, galactose, or other monosaccharides by a combined action of different types of glycosyltransferases (Laws *et al.,* 2001). Four major consecutive steps of EPS biosynthesis in LABs involve sugar transport into the cytoplasm, synthesis of sugar-P, polymerization of repeating unit precursors, and finally EPS export outside the cell (Laws *et al.,* 2001). So, besides the early-mentioned causes, prebiotic could have also provided additional energy to potentiate EPS biosynthesis, thus consequently improving firmness and viscosity properties.

A similar effect was demonstrated by Tamime (2005) for the protein content. Also, M.N.Oliveira *et al.,* (2001) observed an increase in firmness or cohesiveness from 0.12 to 0.20 N when milk was supplemented with whey and from 0.71 to 0.86 N with milk proteins. The above enforcing effect of storage at 4 °C is in agreement with the viscosity increase observed by several authors mainly during the first week of storage (Martin *et al.,* 1999).

The results showed that adding carrot pulp to yogurt significantly increased antioxidant activity. Up to 6% of carrot pulp increased the antioxidant activity up to 52.5 % on 7 days of storage and up to 55.8 % after 14 days (Table 4). The increased addition of carrot pulp to yogurt can increase the antioxidant activity. Research on yogurt with 1% ginger powder (Felfoul *et al.,* 2017) showed that the (RSA) % increased to 95.56% after 14 days of storage. Larasati *et al.* (2018) noted that more than 0.5% antioxidant activity of yogurt with red ginger extract was 71.60%. Then we can say to the carrot is one of the important root vegetables rich in bioactive compounds like total carotenoids, phenolics compounds, and dietary fibers with appreciable levels of several other functional components having significant health-promoting properties. The consumption of carrot and its products is increasing steadily due to its recognition as an important source of natural antioxidants having anticancer activity (Krishan Datt *et al.,* 2012). Two major classes of phenolics are hydroxycinnamic acids and para-hydroxybenzoic acids (Babic *et al.,* 1993). Further, Zhang and Hamauzee (2004) have studied the phenolic compounds, their antioxidant properties and distribution in carrot and found that it contained mainly hydroxycinnamic acids and derivatives.

Oviasogie *et al.* (2009) have reported that the total phenolic content in carrot is 26.6 ± 1.70 µg/g. Epidemiological studies have demonstrated or at least suggested numerous health effects related to probiotics, prebiotics and natural antioxidants (Grajek *et al.,* 2005).

Table 4: Antioxidant activity of carrot yoghurt products during storage.

Parameters	Radical scavenging capacity (RSA) %			
	Control	2%	4%	6%
Zero-time	10.5	34.5	41.3	46.3
7 days	11.3	33.2	44.2	52.5
14 days	9	36.4	46.3	55.8

3.1. Water holding capacity (WHC)

The addition of carrot pulp 2%,4%,6% resulted in a significant increase of the yogurt's WHC to 59.7, 58.3 and 61.3 % compared with control 57.7 % as showed in (Table 5) within storage period. Carrot pulp contains 7.7 % carbohydrate and 2.5 crude fiber (wet weight basis) that can bind water during yogurt processing; fiber can form a gel and bind water during the production of yogurt, as highlighted by Lobato-Calleros *et al.,* (2014). In the present study, the addition of carrot concentrations to yogurt caused an increase WHC. A greater WHC will reduce the quantity of water lost in carrot yoghurt at high temperatures during handling and storage times.

The duration of storage had a significant effect ($p<0.05$) on increasing the WHC of the yogurt supplemented with carrot (table 5). Protein in the milk may increase the yogurt gel network density and result in WHC (Krasaekoopt *et al.,* 2004).

Sodini *et al.,* (2004) reported that the casein may favor micellar interactions, resulting in decreased pore dimensions of the matrix and increased density.

Table 5: Water holding capacity (WHC) of carrot yoghurt products.

Parameters	Water holding capacity (WHC)%			
	Control	2%	4%	6%
Zero-time	56	55	54	57
7 days	58	62	61	63
14 days	59	62	60	64

The data in Table (6) shows the effect of storage on yoghurt made from cows' milk supplemented with different levels of carrot pulp (2%, 4% and 6%) stored at 4 °C for 1, 7 and 14 days.

The pH gradually decreased during fermentation of yoghurt. The average pH of control yoghurt at Zero-time was 4.7 ±0.05 which was higher compared with that reported by Frank (1970) who gave a value of 4.4 in control yoghurt, and higher than that of the different carrot yoghurt samples 2%, 4% and

6%, which had pH values of 4.6 ±0.06, 4.6 ±0.07 and 4.5 ±0.09, at Zero-time respectively. The pH in control decreased after 7 days and 14 days of storage to 4.5 ±0.03 and 4 ± 0.06 respectively. The pH of carrot yoghurt samples 2%, 4% and 6%, decreased after 7 days of storage to 4.4 ±0.08, 4.3 ±0.01 and 4.05 respectively. The decrease continued after 14 days of storage and reached 4.05 ±0.02, 4.03 ±0.004 and 4.0 ±0.04 in carrot yoghurt samples 2%, 4% and 6%,, respectively.

On the other hands, the reduction in pH causes the casein micelles to reach an isoelectric point during fermentation (pH 4.6); it fosters the solubilization of colloidal calcium, enhances hydrophobia interactions, and thus causes casein micelles to form three-dimensional compounds, also the casein micelles play an essential role in the coagulation of milk acid (Phadungath, 2005)

On the other hands, the titratable acidity (expressed as lactic acid %) increased during fermentation of yoghurt. The titratable acidity % of control yoghurt at Zero-time was 1.5 ±0.05% which was slightly higher than that reported by Frank (1970) who reported a value of 1.2% titratable acidity in cow's milk yoghurt. The obtained titratable acidity of control yoghurt was lower than that of carrot yoghurt samples 2%, 4% and 6%, which averaged 1.8 ±0.06%, 1.7 ±0.07% and 1.6 ±0.09%, respectively**.** The increase in acidity of control yoghurt reached 1.6 ±0.04% at 7 days of storage while in carrot yoghurt samples 2%, 4% and 6%, the acidity averaged 1.4 ±0.08, 1.8 ±0.06% and 1.9 ±0.7 %, respectively. The increase in acidity continued until 14 day of storage, it reached 2 ±0.06 % in control yoghurt and 1.8 ±0.68%, 2.03 ±0. 3% and 2.6 ±0.8 % in carrot yoghurt samples 2%, 4% and 6%, respectively.

Osman (2004) reported a similar trend of results, and this may be due to the presence of lactic acid as a result of conversion of lactose by the microorganisms (*Lactobacillus delbrueckii* subsp*. bulgaricus* and *S. thermophilus* (Donkor, *et al.,* 2006). In the present study, the increase of acidity and decreaseing in pH value was in line with the increasing in the carrot pulp added.

Table 6: pH and acidity carrot yoghurt samples during storage periods.

Storage periods	Zero-time				7 day				14 day			
Samples	Control	2%	4%	6%	Control	2%	4%	6%	Control	2%	4%	6%
pH	4.7± 0.05	4.6± 0.06	4.6± 0.07	4.5± 0.09	4.5± 0.03	4.4± 0.08	4.3± 0.01	4.05± 0.00	4.0± 0.06	4.05± 0.02	4.03± 0.004	4.0± 0.04
Acidity (%)	1.5± 0.05	1.8± 0.06	1.7± 0.07	1.6± 0.09	1.6± 0.04	1.4± 0.08	1.8± 0.06	1.9± 0.90	2.0± 0.06	1.8± 0.68	2.03± 0.3	2.6± 0.8

Results of color analysis are shown in table (7) The control yoghurt had lower a* and b* which represent redness and yellowness and this was in agreement with the values obtained by Hashim *et al.* (2009). With the addition of carrot pulp 2, 4,6%, the lightness decreased while the redness and yellowness increased. This was due to the color of carrot which is orange. With increase in carrot pulp, the redness and yellowness increased showing the increase in the concentration of color pigments with increase in carrot pulp. The yoghurt with 6% carrot pulp had high a* and b* values, whereas were 4.44, 16.16 respectively, compared with control yoghurt -0.8, 6.96 of a* and b* values, as showed in table (7 .

Table 7: Color analysis of carrot pulp and carrot yoghurt samples.

Treatments	Color		
	L *	a*	b*
Raw carrot pulp	34.12a	29.75a	43.22a
Control yoghurt	92.33a	-0.8a	6.96a
carrot yoghurt:2%	89.22b	4.44b	16.16b
carrot yoghurt:4%	90.40b	8.33b	18.22b-c
carrot yoghurt:6%	87.14c	11.87c	18.98c

The results of microbial analysis for current trial are shown in Table 8, Yeast, mould were not detected, the average value of yeast and mould count of carrot yoghurt were not found, in spite the acceptable value of yeast and mould count in yoghurt should not be more than 100/g according to PFA (2004).

On the other hand, coliform count in yoghurt incorporated with carrot pulp, all the treatments were subjected to coliform test and it is found to be negative coliform test. This indicated that the yoghurt treatments were free from gas producing organisms. According to PFA (2004) the acceptable value of coliform count in yoghurt should not be more than 10/g. Contamination with the coliform organism is a common problem is the industry and they are completely undesirable in any products.

Table 8: Viable cell counts (*Streptococcus thermophilus*, *Lactobacillus bulgaricus*), yeast, mould and coliform count (c.f.u/ml) of yogurt samples during storage periods.

Storage days	Zero-time				7 day				14 day			
Samples	Control	2%	4%	6%	Control	2%	4%	6%	Control	2%	4%	6%
***Streptococcus thermophilus* cfu/ml**	3.1×10^4	3.4×10^4	3.6×10^4	3.8×10^4	3.6×10^4	4.4×10^4	4.7×10^4	4.9×10^4	3.7×10^4	4.6×10^4	4.9×10^4	5.4×10^4
***Lactobacillus bulgaricus* cfu/ml**	8.1×10^5	8.0×10^5	8.4×10^5	9.2×10^7	8.4×10^6	8.7×10^6	8.8×10^6	9.1×10^6	1.7×10^7	5.0×10^7	7.7×10^7	8.5×10^7
Yeast and mould (cfu/ml)	-	-	-	-	-	-	-	-	ND	ND	ND	ND
Coliform count (cfu/ml)	-	-	-	-	-	-	-	-	ND	ND	ND	ND

Also table (8) shows the microbiological characteristics of control yoghurt and carrot yoghurt at different storage periods. *Streptococcus thermophilus* in control yoghurt was 3.1 x 10^4 c.f.u/ml at zero-time. On the other hand, *Streptococcus thermophilus* in carrot yoghurt samples C1, C2 and C3 slightly increased to 3.4 x 10^4 cfu/ml, 3.6 x 10^4 cfu/ml and 3.8 x10^4 cfu/ml, respectively. However, *Streptococcus thermophilus* continued to increase in the 7th day of storage to 4.4 x 10^4 cfu/ml, 4.7 x 10^4 cfu/ml and 4.9 x 10^4 cfu/ml in carrot yoghurt samples C1, C2 and C3 and 3.6 x10^4 cfu/ml in the control yoghurt. It also continued to increase after 14 days of storage to 4.6 x 10^4 cfu/ml, 4.9 x 10^4 cfu/ml and 5.2 x 10^4 cfu/ml in carrot yoghurt (C1, C2 and C3) and 3.8 x 104 cfu/ml in the control yoghurt.

The *Lactobacillus bulgaricus* bacteria of control yoghurt at zero-time was 8.1 x 10^5 cfu/ml and increased to 8.4x10^5, 9.2 x 10^7 cfu/ml in carrot yoghurt samples C2 and C3, respectively.

The *Lactobacillus bulgaricus* continued to increase in the 7th day of storage to 8.7 x 10^6 cfu/ml, 8.8 x 10^6 cfu/ml and 9.1 x 10^6 cfu/ml in carrot yoghurt samples C1, C2 and C3 and 8.4 x10^6 cfu/ml in the control yoghurt. It also continued to increase after 14 days of storage to 5.0 x 10^7 cfu/ml, 7.7 x 10^7 cfu/ml and 8.5 x 10^7 cfu/ml in carrot yoghurt (C1, C2 and C3) and 1.7 x 10^4 cfu/ml in the control yoghurt. Whereas St. viable cell counts was significantly influenced by the type of compounds of carrot pulp and the storage duration. Carrot pulp stimulated the growth of the Lb as the lactic bacteria, whose counts dramatically increased from 3.6,4.7 and 5 CFU/mL as average, either at zero-time, 7 day and 14 days of storage at 4 °C of all level additions, respectively, compared with control yoghurt at zero-time, 7 day and 14 days of storage at 4 °C. This result not only confirms the positive effect of carrot pulp on viable cell counts already reported in other studies about prebiotic (Donkor *et al.,* 2007; Oliveira *et al.,* 2011), but also suggests the occurrence of a synergistic effect between St and Lb microorganisms with carrot pulp as a prebiotic. As showed results of lactic acid bacteria of carrot yoghurt samples exhibited higher counts than the control. Such an elevation of lactic acid bacteria can be explained on the basis that presence of carrot pulp in the medium of bacteria represents pivotal source of nutrients and thereby increase the growth rate of bacteria.

Table (9) Summarizes the mean scores for sensory attributes as determined by panelists for control and carrot yoghurt samples. The organoleptic properties in both control and carrot yoghurt samples after manufacture were good. There was no significant differences (P>0.05) observed between control and yoghurt with 2, 4 and 6% carrot pulp at zero time. The flavor and odor were pleasant and sweet, appearance was normal. During cold storage the scores increased with increase in the percentage of added carrot pulp. This can be attributed to the sweet taste of manufactured carrot yoghurt. After 2 weeks of storage, the organoleptic properties of yoghurt with different carrot pulp concentration become even better particularly yoghurt with 6 % carrot pulp, total scores at end storage period was 95.4 compared with control yoghurt which was 81.3. However, yoghurt with 6 and 4% and 2 % carrot pulp can be considered acceptable. The addition of 6 % carrot pulp to yoghurt showed to be the best concentration as it got the highest evaluation marks during the storage period. It implies no unpleasant

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aftertaste, a pleasant level of acidity and pleasing balance of flavor during the storage period and without any spoiled by mold and yeast of all samples, and showed shelf life within 14 days. The result indicated that the panelists mostly preferred carrot yoghurt supplemented with 6% carrot pulp.

On the other hand, According to Teshome *et al.,* (2017) Addition of the fruit to yoghurt with optimal level improved sensory attributes and physic-chemical properties of yoghurt.

Table 9: Mean scores for sensory evaluation of carrot yoghurt products.

Sensory attributes	Samples	Storage period			Mean
		Zero-time	Day 7	Day 14	
Appearance (10)	Control	8.3	7.4	7.1	7.6
	2%	7.8	7.3	6.3	7.13
	4%	8	7.3	6.8	7.33
	6%	8.7	7.7	7.5	8
Body & texture (30)	Control	27.5	25.8	24	25.76
	2%	27	25.8	23.3	25.36
	4%	26.8	26	23.6	25.46
	6%	27.6	27.8	29.3	28.23
Flavor (60)	Control	54	53.5	50.2	52.7
	2%	55.5	59.5	53.6	56.2
	4%	55	59.8	51.3	55.36
	6%	59.2	59.7	58.7	59.2

Data are the mean of 10 panelists.

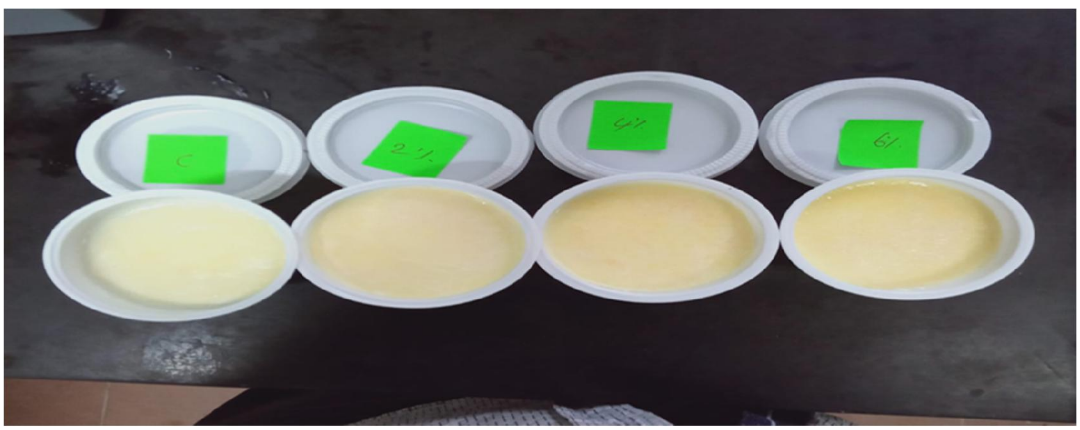

Fig. 4: Shows photo of plain or control yoghurt and carrot yoghurt.

4. Conclusions

The present work dealt with the effect of carrot pulp as a prebiotic and symbiotic interactions between *S. thermophilus*, *L. bulgaricus* cultures, to improve firmness and viable cell counts of caw fermented milk. Results obtained using *S.thermophillus*, *L. bulgaricus* culture, in combination with carrot, point out carrot as an interesting prebiotic to get functional dairy products. The prebiotic concept has in fact emerged and termed as non-viable and non-digestible ingredients that stimulate gut macrobiotic, prebiotic are generally metabolized by health beneficial bacteria and improve immunity to fight against pathogenic organisms.

Fortifying yoghurt or dairy products with carrot pulp is of great interest to improve the functionality of foods with health benefits. The addition of carrot pulp to yoghurt would complement its healthy characteristics. Further researches on the effect of carrot pulp on the nutritional composition will help to show if the nutrients from the carrot pulp will still be available after combining the carrot pulp with yoghurt. There is also a need to study the effects of carrot powder on the shelf life of yoghurt by using another lactic acid bacteria.

Finally, use of carrot pulp in preparation of yoghurt from cows' milk resulted a product with good quality of firmness and viable account cell plus prolonged shelf life. In addition, the prepared yoghurt samples were highly accepted of pH, and acidity with all ratios 2,4 and 6 % carrot pulp. It is highly recommended to encourage dairy industry to use carrot pulp in yoghurt in production of dairy products.

Along with increasing of carrot concentrations in fortified yoghurts, fiber content elevation subsequently reflected on significantly higher values of springiness, and descendant hardness values that produced "soft" yoghurts as described by panelists in sensory evaluation. According to obtained results, carrot pulp is recommended as food additive in food industry due to its unique structural, physical and compositional properties and nutritive value as a bioyogurt. This outcome would provide useful information to manufacturers contributing innovative applications of carrot pulp as source natural, thermal stable thickening and gelling agent, and as healthy positive effect for consumers.

We suggested the necessity of using carrot pulp in preparing yoghurt as a means to secure preservation and extend its shelf life while preserving its vital value, and this would contribute to the development of dairy production technology. on the other hands fortifying yoghurt or dairy products with carrot pulp is of great interest to improve the functionality of foods with health benefits. The addition of carrot pulp to yoghurt would complement its healthy characteristics. More research is needed to investigate the therapeutic and healthy effects of carrot yoghurt.

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