Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2017, 9(8):71-79
(http://www.derpharmachemica.com/archive.html)

Utilization of Novel Nontoxic Antimicrobial Microcrystalline Cellulose in Manufacture of Stirred Yoghurt

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ABSTRACT

Microcrystalline cellulose from rice straw bleached pulp was prepared using solar energy then, it modified to synthesize novel nontoxic antimicrobial cellulose (MAMC) derivative which utilized in the manufacture of stirred yoghurt. Different concentration of (MAMC) was used and compared with stirred yoghurt (control). Microbiologically, chemically, organoleptic and physical properties of resultant stirred yoghurt were studied. Also, the antimicrobial activity of the different concentration of MAMC was assayed against bacterial and fungal pathogenic strains. Results revealed that MAMC was shown good effect against all the pathogenic strains especially at 1.00% concentration which the diameter of inhibition zone ranged from 10 to 15 mm. Addition of MAMC had slightly increased on the viability of Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus and Bifidobacterium bifidum. Moreover, all treatments free from coliform, yeast and mold compared with control when fresh and during cold storage. Moreover, Additive MAMC impose various degrees of inhibition against the growth of some pathogenic and fungi that cause food contamination and spoilage. The acidity and SN/TN of stirred yoghurt treatments increased with prolonged storage period but declined with increasing the ratios of MAMC addition. Sensory evaluation showed that 0.5% of MAMC was the best concentration that had a highest total score during storage. It was recommended that use of MAMC as natural preservatives of stirred yoghurt improve the overall acceptability at ratio 0.50% and extended the shelf life of this product.

Keywords: Natural preservatives, Green chemistry, Stirred yoghurt, Antimicrobial, Microcrystalline cellulose

INTRODUCTION

Microcrystalline cellulose is an insoluble hydrocolloid derived from cellulose typically found in fruits and vegetables. During processing Microcrystalline cellulose forms a thixotropic three dimensional network that provides suspension of heavy particulates, without gelling or inhibiting flow. Microcrystalline cellulose has many applications in pharmaceuticals and foods preparation. It is a naturally derived stabilizer; texturize agent and fat replacer [1]. It is used extensively in reduced fat salad dressing, numerous dairy products including cheese, frozen desserts and whipped topping and bakery products. The microcrystalline cellulose molecules are made up of a chain of about 250 glucose molecules. Thus, we prepared microcrystalline cellulose utilizing bleached pulp obtained via bio-chemical solar pulping process of rice straw [2]. The obtained bleached pulp was degraded to its corresponding microcrystalline cellulose using solar acid hydrolysis [3].

Yogurt is definite as the product that manufactured from milk with or without the adding some natural derivative of milk, as skim milk powder, whey protein or cream, with a gel composition that results from the coagulation of the milk proteins, due to the lactic acid secreted by bacteria cultures. The most popular types of yogurt commercially found are set type yogurt and strained yogurt, though recently frozen yogurt and drinking yogurt have become accepted.

The fermentation process is the mainly stage of yogurt manufacture. During this stage, the yogurt curd is formed, and its textural characteristics and distinct flavor are developed [4,5]. For the fermentation of yogurt, it should contain two live bacterial strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*.

However, yogurt starter cultures may contain other probiotic microorganisms like *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus lactis*, *Bifidobacterium longum*, *Bifidobacterium bifidum*. So that, fermented dairy products, and especially yogurt, are best carrier for probiotic cultures to go through the human digestive system and make certain their survivability through the stomach [6].

In continuation to our program aim to synthesized new biologically active compounds utilizing active methylene precursors [7,8]. The aim of this work is studying the feasibility of using our novel nontoxic MAMC as a green foods preservative in manufacture of stirred yoghurt.

MATERIALS AND METHODS

Materials

Fresh cow's full cream milk was procured from Animal Production Research Institute, Agriculture Research Center, and Dokki, Egypt. Rice straw wastes were obtained from Nobaria farm which belong to National Research Center. Male and female Albino Wistar rats were obtained from animal breeding house at the National Research Center, Egypt.

Pathogenic strains

Bacillus cereus B-3711 and *Aspergillus flavus* 3357 were provided by the Northern Regional Research Laboratory Illinois, USA (NRRL). *Listeria monocytogenes* 598 was provided by the Department of Food Science, University of Massachusetts, Ambert MA, and USA. *Escherichia coli* 0157: H7 and *Staphylococcus aureus* were isolated and serologically identified by dairy microbiological Lab., National Research Center. *Aspergillus niger* was obtained from Department of Microbiology, Swedish University of Agricultural Sciences. The bacterial pathogenic strains were activated in trypton Soya broth, incubated at 37ºC for 24 h. Mold strains were activated in Malt extract broth, incubated at 25ºC for 72 h.

Lactic acid strains

Lactobacillus delbrueckii subsp. *bulgaricus* Lb-12 DRI-VAC, provided by Northern Regional Research Laboratory. Illinois, USA. *Streptococcus thermophilus*CH-1 obtained from Chr. Hansens's Lab., Denmark, *Bifidobacterium bifidum* was obtained from Chr. Hansen's Lab., Denmark.

Preparation of microcrystalline cellulose

The microcrystalline cellulose was fabricated via bio chemical processing of rice straw was done according to Nawwar et al. [9] then the obtained unbleached pulp was bleached and converted to its corresponding microcrystalline cellulose according to Nawwar and Mohamed [10]. It modified to synthesize novel nontoxic antimicrobial cellulose derivative [11].

Animals experimental according to Nawwar

Table 1 included the animal experimental data as well as the housing conditions of each experimental and the administration dose [11].

Table 1: Animals experimental data

Species	Rat
Strain	Albino Wistar rats
Number	10
Age	8-10 weeks
weight	130 ± 10 g
Sex	male/female
Housing conditions of each one experimental	
Temperature	20 ± 2°C
Relative Humidity	40-60%
Lighting	12 h dark / light cycle
Diet type	Standard pellet diet
Water source	Tap water
Administration dose	
Dose	Starting dose
Dosing volumes	0.5 ml/rat
Starting dose	3000 mg/kg b.wt

Acute oral toxicity

Acute toxicity studies with A have been carried out by Chemical Industries Research Division, National Research Centre, Egypt. According to Economic Co-operation Development (OECD) Guidelines and Good Practice, acute oral toxicity was conducted in accordance with the OECD guideline number 423 (OECD, 1996; adopted December 2001).

Acute dermal toxicity

Acute dermal toxicity was conducted in accordance with the OECD guideline number 402 (OECD, 1987; adopted February 1987).

Acute dermal irritation/corrosion

Acute dermal irritation/corrosion was conducted in accordance with the OECD guideline number 404 (OECD, 2002; adopted April 2002).

Assessment of the antimicrobial activity of MAMC

The disc diffusion method according to Jagadeesh et al. [12] was used to determine the antimicrobial activity of the four different concentration of MAMC against pathogenic strains. For pathogenic bacteria, 0.1 ml (approximately 10^9 cells /ml) of the tested microorganisms were spread on the entire surface of the Petri dish containing plate count agar medium using a sterile swab. Portion of this MAMC where diluted by distilled water to give final concentration of 0.25, 0.50, 0.75 and 1.00% (resembling the concentration of MAMC in the stirred yoghurt). Twenty micro liter of each concentration impregnated on different sterile paper discs (What man, 1.6 mm) and placed on the surface of plate count agar medium in Petri dishes. The plates were incubated at 37.0ºC for 24.0 h. After the incubation period the inhibition zones around the paper discs were measured in millimeters.

Preparation of stirred yoghurt

Fresh cow`s full cream milk (4% fat, 13.16% total solid, 6.16 pH value, 0.26% titrataple acidity and 3.15% total protein) heated at 80 ± 1ºC for 30 s then cooled and adjusted to 42ºC according to Marshall and Rawson [13], The milk was divided into five treatments.

Control, inoculated with (1:1%) *S. thermophilus* and *L. bulgaricus* and *B. bifidum* (1%).

Treat 1, was inoculated with (1:1%) *S. thermophilus* and *L. bulgaricus* and *B. bifidum* (1%) then added 0.25% of MAMC.

Treat 2, was inoculated with (1:1%) *S. thermophilus* and *L. bulgaricus* and *B. bifidum* (1%) then added 0.50% of MAMC.

Treat 3, was inoculated with (1:1%) *S. thermophilus* and *L. bulgaricus* and *B. bifidum* (1%) then added 0.75% of MAMC.

Treat 4, was inoculated with (1:1%) *S. thermophilus* and *L. bulgaricus* and *B. bifidum* (1%) then added 1.00% of MAMC.

The samples were transferred into 40 ml plastic cups and incubated at 42ºC for 2 to 4 h until coagulation, after which the cups were stirred with glass rod and stored at 5 ± 1ºC for 15 days. The produced stirred yoghurt treatments were analyzed when fresh, and after 5, 10 and 15 days of storage.

Another separated contaminated samples with a cocktail of four pathogenic strains obtained by mixing the same population (~10^6 CFU/ml) of the different strains of each microorganism. The inoculated milk was divided in the five treatments as mentioned before.

Chemical analysis

Fresh stirred yoghurt samples were chemically analyzed for Total Solids (TS), fat, Titrataple Acidity (TA) and ash content according to AOAC [14]. The pH values were measured using a digital laboratory Jenway 3510 pH meter, UK. Bibby Scientific LTD. Stone, Stafford shire, ST 15 OSA. Total Nitrogen (TN) and soluble nitrogen (S.N) contents were determined using the semi micro-Kjeldal method as mentioned by Ling [15]. The carbohydrate values were obtained by calculation. All of the analyses were run in triplicate**.**

Apparent viscosity of stirred yoghurt (c.P.s)

Stirred yoghurt mix samples were gently stirred 5 times in clockwise direction with a plastic spoon prior to viscosity measurements. Apparent viscosity was measured at 25ºC using a Brookfield digital viscometer (Middleboro, MA02346, and USA). The sample was subjected to shear rates ranging from 10 to 120 S-0 for upward curve. Viscosity measurements were expressed as centipoises (c.P.s) and were performed in triplicate.

Microbiological analysis in stirred yoghurt

L. bulgaricus count was determined using MRS agar [16]. The plates were incubated at 37ºC for 48 h under anaerobic condition. *S. thermophilus* counts were determined using M17 agar [17]. The plates were incubated at 35ºC for 48 h. The count of *B. bifidum* was done according to Vinderola and Reinheimer [18] using modified MRS agar (Oxoid) supplemented with 0.05% L. Cysteine-HCL (Merck, Germany). The plates were incubated at 37ºC for 48 h under anaerobic conditions. Yeasts and Molds count were enumerated using potato dextrose agar acidified to pH 3.5 with sterile lactic acid solution (10%) [19]. Plates were aerobically incubated at 25ºC for 4 days. Coliform bacterial counts were enumerated using violet red bile agar medium [20]. The plates were incubated at 37ºC for 18 h. Colony forming units were counted (cfu/ml) and the results expressed as their log10 values.

Microorganisms counts in contaminated stirred yoghurt

The pathogenic bacteria were detected using Listeria Selective Agar medium for *Listeria monocytogenes*, Sorbitol MacConky Agar Medium for *Escherichia coli* O157 [21], Baird Parker Agar with egg yolk for *Staphylococcus aureus* [22] and potato dextrose agar acidified to pH 3.5 with sterile lactic acid solution (10%) for *Aspergillus flavus* [19].

Sensory evaluation

Sensory evaluation of different treatment of stirred yoghurt was carried out according to Nelson and Trout [23], for flavor (40 points), body and texture (40 points), and appearance (20 points). The Scoring panel consisted of the 12 staff member of Dairy Department, National Research Centre.

Statistical analysis

The data were analyzed according to Statistical Analysis System Users Guide SAS [24], (SAS Institute, Inc., USA). Separation among means in triplicates was carried out using ANOVA test followed by Duncan multiple tests. All Tables result data analysis in two ways. Except chemical composition and sensory evaluation, Tables data analysis in one way.

RESULTS AND DISCUSSION

Toxicological studies of MAMC

Acute oral toxicity

No mortality was noted of animals following single oral treatment of 3000 mg of MAMC The acute oral LD_{50} to rats of both sexes was found to be greater than 3000 mg/kg b.wt (rat: >3000 mg/kg b.wt).

Acute dermal toxicity

No mortality and no signs of toxicity were noted in any of the animals following the dermal application of 3000 mg/kg b.wt of MAMC. According to the above results, the acute dermal LD_{50} to rats was found to be greater than 3000 mg/kg b.wt.

Acute dermal irritation/corrosion

No irritation/corrosion and no signs of toxicity were noted in any of the animals following the dermal application of 3000 mg/kg b.wt of antibacterial cellulose derivative MAMC. Based on the above mentioned results MAMC could be used in deferent industrial and food applications for the subsequent reasons; biodegradable, facial synthesis in good yield and prepared from the cellulosic pulp of many agricultural wastes as rice straw.

Zone of inhibition growth for pathogenic strains by different ratios of MAMC

The antimicrobial activity was assessed using the agar disc diffusion method. Table 2 showed that these MAMC had various degrees of inhibition against the growth of some pathogenic and fungi that cause food contamination and spoilage. From the table it is clear that MAMC were most effective against *L. monocytogenes* especially at high concentration (1%). Moreover, from the table found that *A. niger* more resistant to MAMC at different concentration. MAMC have antimicrobial activity against studied Gram- positive bacteria, Gram-negative bacteria and fungi. The diameter zone of inhibition ranged 5.00 to 10.00 for gram positive bacteria and ranged 4.00 to 15.00 for gram negative bacteria; moreover the diameter of zone recorded 4.00 to 12.00 for fungi. The zone of inhibition decreased with the lowest concentration of MAMC (0.50%) and not detected any inhibition zone for 0.25% MAMC. This result came in accordance with a previous study which indicated that rice straw nano fibrillated cellulose films showed higher antimicrobial properties against *Staphylococcus aureus*, *Escherichia coli* and *Saccharomyces cervisiae* [25]. So, the antimicrobial activity of MAMC that was shown in Table 2 may suggest potential application as natural preservatives when they get incorporated into some dairy products.

Table 2: Zone of inhibition growth for pathogenic strains by different ratios of MAMC

Pathogenic strains	0.25%	0.50%	0.75%	1.00%
	Diameter of inhibition zone (mm)			
Escherichia coli	N.D	$4^{Bc} \pm 0.12$	$9^{Bb} \pm 0.014$	$12^{Ba} \pm 0.11$
Bacillus cereus	N.D	$5^{Ac} \pm 0.08$	$7^{Cb} \pm 0.08$	$11^{BCa} \pm 0.14$
Staphylococcus aureus	N.D	$4^{Bc} \pm 0.08$	$8^{BCb} \pm 0.08$	$10^{Ca} \pm 0.08$
Listeria monocytogenes	N.D	$5^{Ac} \pm 0.12$	$11^{Ab} \pm 0.12$	$15^{Aa} \pm 0.14$
Aspergillus niger	N.D	N.D	$7^{Cb} \pm 0.08$	$10^{Ca} \pm 0.14$
Aspergillus flavus	N.D	$4^{Bc} \pm 0.08$	$10^{Ab} \pm 0.08$	$12^{Ba} \pm 0.15$

Data expressed as mean of 3 replicates ± standard error. Means in the same row showing the same small letters are not significantly different ($P \leq 0.05$). Means in the same column showing the same capital letters are not significantly different ($P \leq 0.05$).

Chemical composition of stirred yoghurt contains different ratios of MAMC

Data presented in Table 3 explain the chemical composition of the stirred yoghurt fortified with MAMC. Addition of MAMC had significant effect on T.S, moisture, protein, fat, carbohydrates and ash. The highest values of T.S, carbohydrates and ash noted with treatments fortified with 1.00% MAMC. Carbohydrates, T.S and ash ranged from 5.17-6.06%, 13.38-13.91% and 0.58-0.69% respectively, among different treatments and control samples. The addition of MAMC had significant effect on carbohydrates contents of stirred yoghurt treatments compared with control (Table 3). In contrary, the protein, fat and moisture content of stirred yoghurt fortified with MAMC significantly decreased by increasing addition level of MAMC. This decrease may due to that protein and fat were affected by addition level of MAMC. As regards, it could be observed that protein content of stirred yogurt was significantly decreased by approximately 8.26%, 9.37% and 11.02% in stirred yoghurt supplemented with 0.50%, 0.75% and 1.00% MAMC respectively. Also, fat content decreased from 1.75% to 1.00% by addition level compared to control. This variation could be recognized to the high carbohydrates presence as cellulose form in MAMC.

Table 3: Chemical composition of stirred yoghurt contains different ratios of MAMC

Treatments	T.S%	Moisture %	Fat%	Protein%	Ash %	Carbohydrate%
Control	$13.38^{E} \pm 0.057$	$86.62^{A} \pm 0.050$	$4.00^{A} \pm 0.057$	$3.63^{A} \pm 0.057$	$0.58^{D} \pm 0.052$	$5.17^{E} \pm 0.055$
Treat 1	$13.48^{D} \pm 0.054$	$86.52^{B} \pm 0.050$	$3.97^{B} \pm 0.050$	$3.43^{B} \pm 0.055$	$0.62^{C} \pm 0.057$	$5.46^{D} \pm 0.057$
Treat 2	$13.\ 52^{C} \pm 0.055$	$86.48^{C} \pm 0.055$	$3.97^{B} \pm 0.057$	$3.33^{C} \pm 0.057$	$0.65^{B} \pm 0.055$	$5.57^{C} \pm 0.052$
Treat 3	$13.83^{B} \pm 0.050$	$86.17^{D} \pm 0.052$	$3.93^{C} \pm 0.053$	$3.29^{D} \pm 0.054$	$0.68^{A} \pm 0.050$	$5.93^{B} \pm 0.050$
Treat 4	$13.91^{A} \pm 0.057$	$86.09^{E} \pm 0.050$	$3.93^{C} \pm 0.057$	$3.23^{E} \pm 0.050$	$0.69^{A} \pm 0.055$	$6.06^{A} \pm 0.050$

Data expressed as mean of 3 replicates ± standard error. Means in the same column showing the same capital letters are not significantly different ($P \leq 0.05$).

The effect of add different MAMC ratios on chemical composition of stirred yoghurt

Table 4 displays the changes of chemical composition of stirred yoghurt (control) and other experimental products (MAMC) during the storage period at 5 ± 1ºC for 15 days.

There was a significant difference in the acidity and pH values between the controls stirred yoghurt and other treatments. The acidity on all experimental products increased with prolonged cold storage period. The significantly highest values of acidity were gotten with Treat 1 then decreased in other MAMC treatments compared with control samples at the end of storage period. Changes in pH values of all experimental treatments were opposing with acidity values.

Also, SN/TN content increased in all treatments with extended of cold storage period but declined with increasing addition level of MAMC treatments compared to control. This owing to decrease of protein contents in MAMC treatments. Furthermore the TS raised up with high ratios of MAMC that the highest value was recorded with treatment fortified with 1.00% MAMC. Additionally, the TS content in all treatments decreased by progress of storage period.

Table 4: Influence of adding different MAMC ratios on chemical composition of stirred yoghurt storage at 5 ± 1ºC for 15 days

Chemical composition	Storage (days)fresh	Control	Treat 1	Treat 2	Treat 3	Treat 4
T.S	fresh	$13.38^{Ae} \pm 0.050$	$13.48^{Ad} \pm 0.053$	$13.52^{Ac} \pm 0.057$	$13.83^{Ab} \pm 0.057$	$13.91^{Aa} \pm 0.051$
	5	$13.28^{Bd} \pm 0.057$	$13.26^{Be} \pm 0.057$	$13.41^{Bc} \pm 0.053$	$13.82^{Aa} \pm 0.055$	$13.76^{Bb} \pm 0.055$
	10	$12.98^{Cd} \pm 0.050$	$12.68^{Ce} \pm 0.051$	$13.02^{Cc} \pm 0.058$	$13.35^{Bb} \pm 0.057$	$13.53^{Ca} \pm 0.055$
	15	$12.49^{Dd} \pm 0.057$	$12.46^{De} \pm 0.051$	$12.81^{Dc} \pm 0.057$	$13.28^{Cb} \pm 0.057$	$13.41^{Da} \pm 0.057$
T.A	fresh	$0.82^{Db} \pm 0.055$	$0.84^{Da} \pm 0.057$	$0.84^{Da} \pm 0.057$	$0.84^{Da} \pm 0.055$	$0.83^{Dab} \pm 0.057$
	5	$0.92^{Cbc} \pm 0.055$	$0.97^{Ca} \pm 0.055$	$0.93^{Cb} \pm 0.050$	$0.91^{Cc} \pm 0.057$	$0.88^{Cd} \pm 0.051$
	10	$1.04^{Bc} \pm 0.057$	$1.25^{Ba} \pm 0.051$	$1.16^{Ab} \pm 0.057$	$1.00^{Bc} \pm 0.055$	$0.93^{Bd} \pm 0.055$
	15	$1.24^{Ab} \pm 0.051$	$1.30^{Aa} \pm 0.057$	$1.12^{Bc} \pm 0.051$	$1.10^{Ad} \pm 0.057$	$1.00^{Ad} \pm 0.050$
pH	fresh	$4.80^{Ac} \pm 0.051$	$4.84^{Ab} \pm 0.057$	$4.84^{Ab} \pm 0.051$	$4.85^{Ab} \pm 0.057$	$4.87^{Aa} \pm 0.055$
	5	$4.80^{Ab} \pm 0.057$	$4.73^{Bc} \pm 0.050$	$4.81^{Bb} \pm 0.053$	$4.84^{Ba} \pm 0.050$	$4.85^{Ba} \pm 0.050$
	10	$4.77^{Bb} \pm 0.053$	$4.72^{Bd} \pm 0.057$	$4.75^{Cc} \pm 0.057$	$4.83^{Ba} \pm 0.057$	$4.84^{Ba} \pm 0.057$
	15	$4.76^{Bb} \pm 0.050$	$4.66^{Cd} \pm 0.055$	$4.73^{Dc} \pm 0.053$	$4.79^{Ca} \pm 0.055$	$4.79^{Ca} \pm 0.051$
SN/TN	fresh	$0.218^{Aa} \pm 0.050$	$0.189^{Ae} \pm 0.057$	$0.199^{Ac} \pm 0.057$	$0.194^{Ab} \pm 0.055$	$0.183^{Ad} \pm 0.051$
	5	$0.282^{Ba} \pm 0.055$	$0.231^{Bd} \pm 0.050$	$0.237^{Bd} \pm 0.050$	$0.246^{Bc} \pm 0.057$	$0.231^{Bb} \pm 0.051$
	10	$0.339^{Ca} \pm 0.051$	$0.321^{Cd} \pm 0.057$	$0.321^{Cc} \pm 0.050$	$0.251^{Cb} \pm 0.051$	$0.257^{Ce} \pm 0.057$
	15	$0.383^{Da} \pm 0.057$	$0.361^{Dd} \pm 0.055$	$0.371^{Db} \pm 0.051$	$0.375^{Dc} \pm 0.055$	$0.372^{De} \pm 0.050$

Data expressed as mean of 3 replicates ± standard error. Means in the same row showing the same small letters are not significantly different ($P \leq 0.05$). Means in the same column showing the same capital letters are not significantly different ($P \leq 0.05$).

Apparent viscosity of stirred yoghurt (c.P.s) samples

Viscosity values (c.P) are designed as a function of time (s) in Figure 1, to explain the flow behavior of stirred yoghurt samples with different ratios of MAMC. The value of apparent viscosity in the treatments fortified with MAMC (0.25, 0.50, 0.75 and 1.00%) was found to be similar with control treatment without MAMC. Thus, there is no significant affect with the increased of MAMC ratios added in this study. This may due to the low concentrate of MAMC add in this product. Also, it could not be noticed any significant change of apparent viscosity in all treatments during storage period until the end (15 days) (Figure 1).

Viability of *Streptococcus thermophilus* count (Log10 cfu/ml) in stirred yoghurt treatments

The effect of MAMC at different concentrations on growth of *S. thermophilus* count in stirred yoghurt during storage presented in Table 5. The growth of *S. thermophilus* counts was not affected by MAMC. The viable count of *S. thermophilus* slightly increased with the storage period until 10 days of storage and after that slightly decreased at 15 day of storage. The treat 2 and treat 3 have the highest viable count of *S. thermophilus* during storage periods compared with control and other treatments. Also, the effect of MAMC on the activity of the starter culture in the final product was investigated by continuous monitoring by the pH values. These results agree with Nawwar et al. [1] found that adding of Microcrystalline cellulose had no effect on the survive rate of *Lactobacillus delbrueckii* sp. *bulgaricus* and *Streptococcus thermophilus* in yogurt during storage.

Viability of *Lactobacillus bulgaricus* count (Log10 cfu/ml) in stirred yoghurt treatments

The growth of *Lactobacillus bulgaricus* count was not affected by MAMC as in Table 6. In our study indicated that the *L. bulgaricus* count increased during storage until 10 days of storage and decreased after that at 15 days of storage in all treatments. The count in samples that fortified with 0.5% and 0.75% MAMC have the highest counts which the viable count of *L. bulgaricus* reached to 8.70 and 8.80 log10 cfu/ml at 15 days of storage for the same tow treatments respectively, compared with the control and other treatments.

Nawwar et al. [1] noted that the count *L. bulgaricus* remained active and the count more than Log10 7 in treatments of yoghurt that containing microcrystalline cellulose during storage period.

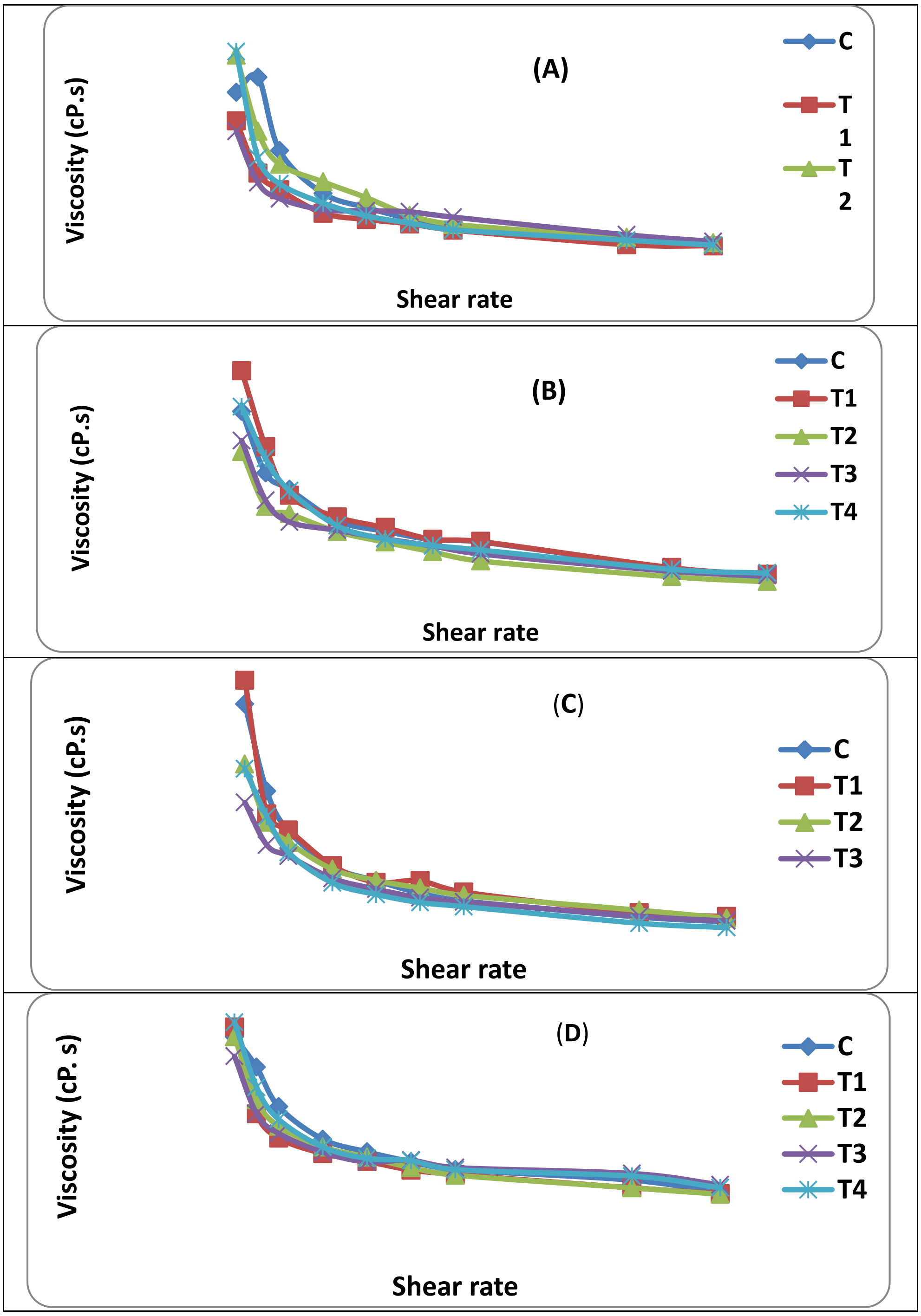

Figure 1: Viscosity of stirred yoghurt samples made with different ratios of MAMC during storage at 5 ± 2°C for 15 days (A) Fresh stirred yoghurt samples (B) Stirred yoghurt after 5 days of storage (C) Stirred yoghurt after 10 days of storage (D) Stirred yoghurt after 15 days of storage

Table 5: Count of *Streptococcus thermophilus* in stirred yoghurt treatments during storage 5 ± 1°C for 15 days

Treatments	Storage periods (days)			
	Fresh	5	10	15
Control	$8.90^{Bb} \pm 0.057$	$9.20^{Ca} \pm 0.057$	$9.15^{Da} \pm 0.028$	$9.10^{Da} \pm 0.088$
Treat 1	$9.00^{Ab} \pm 0.088$	$9.30^{Ba} \pm 0.057$	$9.25^{Ca} \pm 0.025$	$9.25^{Ba} \pm 0.044$
Treat 2	$9.10^{Ac} \pm 0.088$	$9.45^{Aa} \pm 0.024$	$9.35^{Bb} \pm 0.028$	$9.30^{Ab} \pm 0.016$
Treat 3	$9.25^{Abc} \pm 0.025$	$9.40^{Aa} \pm 0.024$	$9.45^{Aa} \pm 0.028$	$9.35^{Ab} \pm 0.044$
Treat 4	$9.10^{Ac} \pm 0.025$	$9.30^{Ba} \pm 0.057$	$9.25^{Cab} \pm 0.016$	$9.15^{Cc} \pm 0.88$

Data expressed as mean of 3 replicates ± standard error. Means in the same row showing the same small letters are not significantly different ($P \leq 0.05$). Means in the same column showing the same capital letters are not significantly different ($P \leq 0.05$).

Table 6: Count of *Lactobacillus bulgaricus* in stirred yoghurt treatments during storage at 5 ± 1ºC for 15 days

Treatments	Storage periods (days)			
	Fresh	**5**	**10**	**15**
Control	$8.50^{Ac} \pm 0.076$	$8.70^{Ba} \pm 0.088$	$8.60^{Bb} \pm 0.054$	$8.35^{Bd} \pm 0.028$
Treat 1	$8.40^{Bb} \pm 0.044$	$8.65^{Ba} \pm 0.028$	$8.70^{Aa} \pm 0.028$	$8.45^{Bb} \pm 0.028$
Treat 2	$8.55^{Ac} \pm 0.028$	$8.80^{Aa} \pm 0.044$	$8.85^{Aa} \pm 0.016$	$8.70^{Ab} \pm 0.044$
Treat 3	$8.45^{Bc} \pm 0.027$	$8.95^{Aa} \pm 0.028$	$8.90^{Aa} \pm 0.044$	$8.80^{Ab} \pm 0.028$
Treat 4	$8.40^{Bb} \pm 0.057$	$8.50^{Ca} \pm 0.028$	$8.45^{Ca} \pm 0.060$	$8.35^{Bb} \pm 0.016$

Data expressed as mean of 3 replicates ± standard error. Means in the same row showing the same small letters are not significantly different ($P \leq 0.05$). Means in the same column showing the same capital letters are not significantly different ($P \leq 0.05$).

Viability of *Bifidobacterium bifidum* count (Log10 cfu/ml) in stirred yoghurt treatments

Viability of *Bifidobacterium bifidum* in stirred yoghurt treatments that fortified with different concentration of MAMC, are shown in Table 7. The results demonstrated that the treatment that contained 0.5% and 0.75% MAMC had the greatest count of *B. Bifidum* during storage period compared with the other treatments and control. Nevertheless, the differences between all treatments that fortified with MAMC and control were slight significant. Conversely, differences tend to be significant among treatments during storage. The detected numerous of *B. bifidum* log10 cfu/ml were increased until 10 days of storage and slightly decreased at the end of storage period in all treatments especially, the treatments that contained 0.25, 1.00% and control. These results confirmed by Nawwar et al. [1] that found the viable count of total bacterial counts in fresh yoghurt supplemented with microcrystalline cellulose was 7.0 log cfu/g and improved to reach 8.9 log cfu/g at the 5th day of the storage, and then slightly decreased at the end of storage.

Table 7: Count of *Bifidobacterium bifidum* in stirred yoghurt treatments during storage at 5 ± 1ºC for 15 days

Treatments	Storage periods (days)			
	Fresh	**5**	**10**	**15**
Control	$8.00^{Cc} \pm 0.057$	$8.40^{Da} \pm 0.028$	$8.50^{Ca} \pm 0.057$	$8.20^{Eb} \pm 0.088$
Treat 1	$8.30^{Ac} \pm 0.028$	$8.65^{Bb} \pm 0.057$	$8.80^{Ba} \pm 0.028$	$8.55^{Cb} \pm 0.028$
Treat 2	$8.25^{Ac} \pm 0.028$	$8.80^{Ab} \pm 0.028$	$9.00^{Aa} \pm 0.057$	$8.85^{Ab} \pm 0.044$
Treat 3	$8.10^{Bd} \pm 0.044$	$8.50^{Cc} \pm 0.057$	$8.90^{Aa} \pm 0.044$	$8.70^{Bb} \pm 0.057$
Treat 4	$8.00^{Cc} \pm 0.057$	$8.35^{Db} \pm 0.028$	$8.60^{Ca} \pm 0.077$	$8.40^{Db} \pm 0.057$

Data expressed as mean of 3 replicates ± standard error. Means in the same row showing the same small letters are not significantly different ($P \leq 0.05$). Means in the same column showing the same capital letters are not significantly different ($P \leq 0.05$).

Detection of mold and yeast count (Log10 cfu/ml) in stirred yoghurt treatments

The quality and shelf life of different fermented dairy products were determined according to the viable count of coliform, mold and yeast during storage period (Table 8). In this study, molds and yeasts count were not detected in fresh all treatments and throughout the storage period. This could be as a result of the antimicrobial activities of MAMC against different pathogenic bacteria, mold and yeast. Conversely, molds and yeasts were detected in control and Treat 1 at 15 days of storage period. The counts of mold and yeast reached to 2.40 and 1.60 log10 cfu/ml in the control (C1) and treat 1, respectively at the end of storage and not detected any mold and yeast growth in other treatments. Moreover, the treatments that fortified with different concentrations of MAMC (0.5, 0075 and 1.00%) remind safe from mold, yeast and coliform up to 15 days but the control become moldy after 15 days of storage, therefore the MAMC could be used as a preservative agent in different fermented products. Notably, coliforms were not detected in all treatment and control during storage period, which indicated the good pasteurization and hygienic condition followed in its production.

Table 8: Count of molds and yeasts in stirred yoghurt treatments during storage at 5 ± 1ºC for 15 days

Treatments	Storage periods (days)			
	Fresh	**5**	**10**	**15**
Control	Nil	Nil	Nil	$2.40^{Aa} \pm 0.028$
Treat 1	Nil	Nil	Nil	$1.60^{Ba} \pm 0.016$
Treat 2	Nil	Nil	Nil	Nil
Treat 3	Nil	Nil	Nil	Nil
Treat 4	Nil	Nil	Nil	Nil

Data expressed as mean of 3 replicates ± standard error. Means in the same row showing the same small letters are not significantly different ($P \leq 0.05$). Means in the same column showing the same capital letters are not significantly different ($P \leq 0.05$).

Microorganisms count (Log10 cfu/ml) in contaminated stirred yoghurt treatments

The effect of different concentration of MAMC in stirred yoghurt treatments containing contaminated pathogenic strains illustrated in Table 9.

It is clear from the Table, the counts of different pathogenic strains gradually decreased by the storage period in all treatments that fortified with different MAMC concentration, but in the control the pathogenic counts remained stable or slight decreased during storage period. As well, the Treat 3 and 4 which contained 0.75 and 1.00% MAMC had higher effect on pathogenic strains and the viable counts of studied pathogenic strains not detected in these treatment at 15 days of storage period compared with control. Also, from these study found that *L. momocytogens* more sensitive to the MAMC at different concentration which found the viable count to this strain decrease from 6.20 to 0.95 Log10 cfu/ml after 10 days of storage and not observed at the end of storage. From these results indicated that MAMC had antimicrobial properties which can be used as a new preservative material to protect fermented dairy products from food born pathogen and spoilage.

Table 9: The effect of adding different ratios of MAMC **in stirred yoghurt treatments stored at 5 ± 1°C for 15 days on the pathogenic strains count (Log cfu/g)**

Microorganisms	Storage period (days)	Control	Treat 1	Treat 2	Treat 3	Treat 4
Escherichia Coli	Fresh	$6.00^{Ac} \pm 0.088$	$6.00^{Ac} \pm 0.057$	$6.20^{Aa} \pm 0.057$	$6.10^{Ab} \pm 0.028$	$6.00^{Ac} \pm 0.033$
	5	$5.80^{Ba} \pm 0.057$	$5.70^{Bb} \pm 0.044$	$4.70^{Bc} \pm 0.044$	$4.00^{Bd} \pm 0.057$	$3.50^{Be} \pm 0.057$
	10	$5.75^{Ba} \pm 0.028$	$5.00^{Cb} \pm 0.028$	$3.00^{Cc} \pm 0.057$	$2.10^{Cd} \pm 0.088$	$1.20^{Ce} \pm 0.014$
	15	$5.55^{Ca} \pm 0.060$	$4.80^{Db} \pm 0.057$	$2.00^{Dc} \pm 0.044$	N.D	N.D
Staphylococcus aureus	Fresh	$5.90^{Ab} \pm 0.028$	$6.00^{Aa} \pm 0.033$	$6.10^{Aa} \pm 0.088$	$5.95^{Ab} \pm 0.029$	$6.00^{Aa} \pm 0.075$
	5	$5.50^{Bb} \pm 0.057$	$5.85^{Ba} \pm 0.028$	$4.00^{Bc} \pm 0.088$	$3.20^{Bd} \pm 0.012$	$2.90^{Be} \pm 0.057$
	10	$5.30^{Ca} \pm 0.057$	$5.00^{Cb} \pm 0.057$	$2.40^{Cc} \pm 0.088$	$2.00^{Cd} \pm 0.028$	$1.50^{Ce} \pm 0.011$
	15	$5.00^{Da} \pm 0.029$	$4.70^{Db} \pm 0.057$	$1.75^{Dc} \pm 0.060$	N.D	N.D
Listeria monoytogenes	Fresh	$6.30^{Aa} \pm 0.066$	$6.10^{Ab} \pm 0.012$	$6.25^{Aa} \pm 0.028$	$6.00^{Ac} \pm 0.088$	$6.20^{Ab} \pm 0.033$
	5	$6.00^{Ba} \pm 0.029$	$5.60^{Bb} \pm 0.057$	$3.65^{Bc} \pm 0.028$	$3.00^{Bd} \pm 0.012$	$2.45^{Be} \pm 0.014$
	10	$5.90^{Ca} \pm 0.060$	$5.45^{Cb} \pm 0.044$	$2.45^{Cc} \pm 0.044$	$1.20^{Cd} \pm 0.010$	$0.95^{Ce} \pm 0.028$
	15	$5.75^{Da} \pm 0.043$	$4.90^{Db} \pm 0.088$	$1.30^{Dc} \pm 0.028$	N.D	N.D
Aspergillus flavus	Fresh	$5.10^{Ab} \pm 0.066$	$5.20^{Aa} \pm 0.088$	$5.00^{Ac} \pm 0.088$	$5.20^{Aa} \pm 0.088$	$5.10^{Ab} \pm 0.088$
	5	$5.00^{Ba} \pm 0.066$	$4.85^{Bb} \pm 0.044$	$3.35^{Bc} \pm 0.028$	$3.00^{Bd} \pm 0.012$	$2.85^{Be} \pm 0.014$
	10	$5.00^{Ba} \pm 0.045$	$4.50^{Cb} \pm 0.057$	$2.50^{Cc} \pm 0.012$	$1.70^{Cd} \pm 0.028$	$1.00^{Ce} \pm 0.014$
	15	$4.70^{Ca} \pm 0.029$	$4.25^{Db} \pm 0.028$	$1.80^{Dc} \pm 0.014$	N.D	N.D

Data expressed as mean of 3 replicates ± standard error. Means in the same row showing the same small letters are not significantly different ($P \leq 0.05$). Means in the same column showing the same capital letters are not significantly different ($P \leq 0.05$).

Sensory evaluation of stirred yoghurt containing different concentration of MAMC

The sensory properties reflect the quality and shelf-life of dairy products. Sensory evaluation for food were flavor, texture and appearance also, it used to observe and score obvious variations that happen over time. Moreover it is suitable when determining the shelf-life of products. As can be observed from Table 10 the results of organoleptic properties shown that addition of MAMC had significant influence on flavor, body texture, appearance and total score. The addition of MAMC to stirred yoghurt improved body and texture and appearance when fresh and through the storage period compared to control treatments. These results are in line with Nawwar et al. [1]. The stirred yoghurt with 0.50% MAMC gained the highest total score either when fresh or after 15 days of storage period. Moreover, all stirred yoghurt samples made with MAMC had acceptable appearance when fresh or after 15 days of storage period contrary the control samples. The addition of MAMC had significant decrease on flavor by increasing ratios of MAMC specifically, in treatments contain high ratio (0.75 and 1.00%) MAMC also, it was appeared a bitter flavor in these high ratios. The sensory properties of all treatments after 15 days of storage decreased compared with fresh treatments and this due to progress of acidity through storage period. The use of MAMC as preservative of stirred yoghurt will improve the overall acceptability at ratios (0.25 and 0.50%) as well as enhance the shelf life of stirred yoghurt. So, it is recommended that MAMC ratio not to exceed 0.50% in this product.

Table 10: Sensory evaluation of stirred yoghurt containing different concentration of MAMC fresh and after 15 days of storage at 5 ± 1ºC

Treatments (fresh)	Flavor (40)	Body& texture (40)	Appearance (20)	Total (100)
Control	$38^{A} \pm 0.055$	$35^{B} \pm 0.050$	$17^{B} \pm 0.052$	$90^{AB} \pm 0.050$
Treat 1	$37^{A} \pm 0.050$	$36^{AB} \pm 0.057$	$18^{AB} \pm 0.057$	$91^{A} \pm 0.057$
Treat 2	$36^{B} \pm 0.051$	$37^{A} \pm 0.053$	$18^{AB} \pm 0.057$	$91^{A} \pm 0.057$
Treat 3	$34^{C} \pm 0.054$	$37^{A} \pm 0.056$	$19^{A} \pm 0.050$	$90^{AB} \pm 0.050$
Treat 4	$33^{C} \pm 0.057$	$37^{A} \pm 0.057$	$19^{A} \pm 0.050$	$89^{B} \pm 0.055$
Treatments (after 15)				
Control	$35^{A} \pm 0.052$	$34^{B} \pm 0.052$	$15^{B} \pm 0.057$	$84^{C} \pm 0.050$
Treat 1	$33^{B} \pm 0.055$	$37^{AB} \pm 0.050$	$16^{AB} \pm 0.050$	$86^{AB} \pm 0.053$
Treat 2	$33^{B} \pm 0.055$	$38^{A} \pm 0.057$	$16^{AB} \pm 0.050$	$87^{A} \pm 0.055$
Treat 3	$31^{C} \pm 0.050$	$38^{A} \pm 0.053$	$17^{A} \pm 0.052$	$86^{AB} \pm 0.054$
Treat 4	$30^{D} \pm 0.057$	$38^{A} \pm 0.053$	$17^{A} \pm 0.052$	$85^{B} \pm 0.050$

Data expressed as mean of 3 replicates ± standard error. Means in the same column showing the same capital letters are not significantly different ($P \leq 0.05$).

CONCLUSION

Utilizing of agricultural wastes to create novel MAMC derived from the cellulosic pulp of rice straw to produce extending shelf life stirred yoghurt. The MAMC achieved good effect against the growth of some pathogenic and fungi which cause food contamination and spoilage. Adding different ratios of MAMC did not have any significant change in the chemicals and physical properties in this product. Also, the counts of lactic acid bacteria had no significant effect contrary counts of pathogenic strains declined during storage period by addition of MAMC. Moreover, use 0.5% concentration of MAMC provides the best sensory evaluation than other treatments. Thus MAMC could be used in different industrial food applications.

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