Sains Malaysiana 53(2)(2024):285-294
http://doi.org/10.17576/jsm-2024-5302-04

Probing the Potential of Water Chestnut Powder *(Trapa bispinosa)* in Improving the Shelf Life of Buttermilk

(Menyelidik Potensi Serbuk Berangan Air (*Trapa bispinosa*) dalam Meningkatkan Jangka Hayat Susu Mentega)

Sadia Rehman Rao[1], Nabila Gulzar[1,*], Muhammad Nadeem[1], Saima Rafiq[2], Fakhara Khanum[5], Shamas Murtaza[4] & Muhammad Ajmal[3]

[1]Department of Dairy Technology, University of Veterinary and Animal Sciences, Lahore, 55300, Pakistan
[2]Department of Food Science and Technology, University of Poonch, Rawalakot, 12350, Pakistan
[3]Food Chemistry Lab, Department of Dairy Technology, University of Veterinary and Animal Sciences, Lahore, 55300, Pakistan
[4]Department of Food Science and Technology, Muhammad Nawaz Sharif University of Agriculture, Multan
[5]Department of Human Nutrition and Dietetics, University of Agriculture, DIKhan

Received: 11 January 2023/Accepted: 20 January 2024

ABSTRACT

Buttermilk, a valuable by-product of butter production, faces challenges in commercialization due to its limited shelf life and susceptibility to fungal growth. This study aimed to explore the use of water chestnut powder (WCP) as a means to extend the shelf life of buttermilk. The investigation involved evaluating the physicochemical properties, antioxidant activity, antifungal properties, and stabilizing effects of WCP in buttermilk. Buttermilk samples were prepared with varying concentrations of WCP (0%, 0.5%, 1%, 1.5%, and 2%) and stored at 4 °C for one month. The analysis of physicochemical properties showed that the concentration of WCP had a significant impact on the protein percentage, ash content, pH, and acidity of the Water Chestnut Buttermilk (WCBM). Assessment of antioxidant activity using the phosphomolybdenum method showed that, on the 21st day of storage, WCBM3 and WCBM4 exhibited total antioxidant capacities of 0.57±0.12 and 0.60±0.32, respectively, compared to the control with a capacity of 0.48±0.07. The antifungal activity of water chestnut powder buttermilk was evaluated using a qualitative method, which demonstrated inhibition of fungal growth. In the control and WCBM1 and WCBM2 treatments, the observed inhibition ranged from 1-4 mm. However, as the concentration of water chestnut powder increased in WCBM3 and WCBM4, the level of inhibition also increased. Textural analysis further indicated the stabilizing effect of WCP on buttermilk. Overall, the incorporation of WCP in buttermilk yielded promising results in terms of enhancing its physicochemical properties, antioxidant activity, antifungal potential, and textural stability. This study highlights the potential of water chestnut as an ingredient to improve the shelf life and quality of buttermilk, creating opportunities for its commercial utilization in the dairy industry.

Keywords: Antifungal; buttermilk; physicochemical; sensory; water chestnut

ABSTRAK

Susu mentega, produk sampingan yang berharga daripada pengeluaran mentega, menghadapi cabaran dalam pengkomersialan kerana jangka hayatnya yang terhad dan mudah terdedah kepada pertumbuhan kulat. Penyelidkan ini bertujuan untuk mengkaji penggunaan serbuk berangan air (WCP) sebagai cara untuk memanjangkan jangka hayat susu mentega. Kajian melibatkan penilaian sifat fizikokimia, aktiviti antioksidan, sifat antikulat dan kesan penstabilan WCP dalam susu mentega. Sampel susu mentega disediakan dengan kepekatan WCP berbeza (0%, 0.5%, 1%, 1.5% dan 2%) dan disimpan pada suhu 4 °C selama satu bulan. Analisis sifat fizikokimia mendedahkan bahawa kepekatan WCP mempunyai kesan yang signifikan terhadap peratusan protein, kandungan abu, pH dan keasidan Air Susu Mentega Berangan (WCBM). Penilaian aktiviti antioksidan menggunakan kaedah phosphomolybdenum menunjukkan bahawa, pada hari penyimpanan ke-21, WCBM3 dan WCBM4 menunjukkan jumlah kapasiti antioksidan masing-masing 0.57±0.12 dan 0.60±0.32, berbanding kawalan dengan kapasiti 0.48±0.07. Aktiviti antikulat serbuk susu mentega berangan air dinilai menggunakan kaedah kualitatif, yang menunjukkan perencatan pertumbuhan kulat. Dalam kawalan dan rawatan WCBM1 dan WCBM2, perencatan yang diperhatikan adalah antara 1-4 mm. Walau bagaimanapun, apabila kepekatan serbuk berangan air meningkat dalam WCBM3 dan WCBM4, tahap perencatan juga meningkat. Analisis tekstur seterusnya menunjukkan kesan penstabilan WCP pada susu mentega. Secara keseluruhannya, penggabungan WCP dalam susu mentega membuahkan hasil yang memberangsangkan daripada segi peningkatan sifat fizikokimia, aktiviti antioksidan, potensi antikulat dan kestabilan tekstur. Kajian ini menyerlahkan potensi berangan air sebagai ramuan untuk meningkatkan jangka hayat dan kualiti susu mentega, mewujudkan peluang untuk penggunaan komersialnya dalam industri tenusu.

Kata kunci: Antikulat; berangan air; deria; fizikokimia; susu mentega

INTRODUCTION

Buttermilk is a by-product obtained when cream or fermented milk is churned at lower temperatures to yield butter (Gebreselassie et al. 2016). Based on annual milk production worldwide approximately, 6.5%-7.0% of total milk produced is used for the preparation of butter that yields around 3.2 million tons/annum of buttermilk as a by-product (Hati, Das & Mandal 2019). Different types of cream can be used to produce buttermilk. One serving of 100 g of buttermilk provides 40 kcal of energy. Typically, traditional fermented buttermilk is composed of 7-10% total solids, which include lactose (3.5-4.9%), lactic acid (0.5%), nitrogenous compounds (2.7-3.8%), and ash (0.6-0.75%). The fat content in this buttermilk ranges from 0.3% to 1.0%, and it contains 90-91% moisture (Narvhus & Abrahamsen 2023). Additionally, it is rich in minerals such as phosphorus, zinc, potassium, magnesium, and calcium, along with vitamins like riboflavin, folic acid, vitamin B, and vitamin B12 (Barukčić, Jakopović & Božanić 2019).

Buttermilk holds significant value due to its nutritional properties and affordability. However, it is often discarded after the production of butter due to its shorter shelf life, tendency for fungal growth, and phase separations. *Penecillium* and *Aspergillus* spp. are the most commonly found fungus in the buttermilk. Currently, pasteurization and chemical preservation methods are being used to increase the shelf life of buttermilk but some of the fungus species can survive certain pasteurization temperature. High temperature pasteurization causes coagulation of proteins in buttermilk that results curdling or clumping and negatively impacts the mouth feel of buttermilk (Morin, Pouliot & Britten 2008). Also, there is a growing trend of natural and clean-label products and consumers prefer to use foods without synthetic additives.

Despite its potential, there are challenges to increase the consumption of traditional buttermilk, including its limited shelf life of approximately one week when stored at 4-7 °C, there is difficulties in achieving consistent quality, and lack of promotion as a beverage (Libudzisz & Stepaniak 2011). In response to these challenges, potential solutions or strategies include increasing the shelf life and promoting buttermilk as a beverage. The commercialization of buttermilk as beverage can be increased by improving its quality characteristics and shelf life using natural preservation ingredients (Dudkiewicz, Hayes & Onarinde 2022).

During the churning process, the milk fat globule membrane (MFGM), which is enriched with phospholipids, ruptures, thus exposing fat globules to atmospheric oxygen. This makes the fat globules susceptible to oxidation reactions, which can decrease the shelf life of buttermilk (Alsaleem 2019).

Although the cream is heat-treated before butter manufacturing, buttermilk remains vulnerable to fungal spoilage (*Cladosporium, Penicillium*) due to the presence of various substances like carbohydrates, organic acids, proteins, and lipids (Huis 1996). This susceptibility can result in economic losses for dairy industries, as it is estimated that 5-10% of global food production is lost to fungal deterioration (Pitt & Hocking 2009). Such losses may arise from the compromised quality of dairy products affected by these fungal contaminants. Nonetheless, a significant number of fungal species are able to thrive in dairy products as they are acidotolerant, xerotolerant, and/or psychrotolerant, and can even withstand chemical preservatives, which are sometimes added to increase product shelf-life. Concerning undesirable species, their presence can cause various types of food spoilage, including visible growth of fungus at the product surface, and the production of metabolites causing off-odors and flavors, and changes in color and texture (Ledenbach & Marshall 2009). In addition to affecting the organoleptic properties, certain spoilage molds like *Penicillium* and *Aspergillus* spp. can also produce mycotoxins (Hymery et al. 2014).

Formation of curdy particles is one of the most prevalent defects in buttermilk, often caused by uneven mixing of the curd and water. This leads to the development of small curd particles in the buttermilk. To avoid this defect, homogenization or the use of a shear pump to shear the buttermilk can be effective. Moving beyond texture issues, buttermilk is often watery in consistency, requiring various extenders and stabilizers to maintain its texture and other physicochemical details (Ghanshyambhai, Balakrishnan & Aparnathi 2015). For this purpose, fibers or complex carbohydrates are the best source and are being used in bulk in the dairy industry these days (Chen et al. 2010). The most important fibers used are obtained from plants or their parts like leaves, stems, fruits, and even seeds. These extenders and stabilizers play a crucial role in maintaining the desired texture and physicochemical properties of buttermilk, ensuring a consistent and high-quality product (Ghanshyambhai, Balakrishnan & Aparnathi 2015).

One of the nutritious fruits is water chestnut commonly known as 'Singhara (*Trapabispinosa*)'. It is an excellent source of carbohydrates and is usually found rooted in water lakes and ponds. It provides thickening, textural, and stabilizing customization of food products so, domestically as well as industrially, it is considered the best additive because of its higher fiber contents and because it can be adjusted with any kind of food (Lutfi et al. 2017). Previous researches proved that water chestnuts have a high antioxidant capacity upto DPPH 16.74 µmol TE/g (Xu et al. 2020). It is indicated that the high level of antioxidant capacity is due to presence of significant concentrations of phenolics and flavonoids (Martínez, Fuentes & Carballo 2022). Water chestnut also had potential inhibitory effect on fungi or molds (Razvy, Kabir & Hoque 2011). Mandal et al. (2011) found antifungal peptide, with molecular mass of 1230 Da from fruits of *Trapa natans* by reverse phase high performance liquid chromatography and named as *Tn*-AFP1. The amino acid sequence of this peptide contained following eleven amino acid residues: LMCTHPLDCSN. Purified *Tn*-AFP1 showed the inhibition effect against *Candida tropicalis* fungus growth.

Due to the significant problems like oxidation, fungal growth and development of curdy particles, it is being lost by the dairy industries in Pakistan. There is a need of ingredient that can overwhelm all these problems. Previous studies on water chestnut showed its antioxidant, antifungal and water stabilizing properties. But its effect on buttermilk shelf life is not being studied earlier. In this study, the antioxidant, antifungal and stabilizing potential of water chestnut in extending the shelf life of buttermilk will be studied.

MATERIALS AND METHODS

PREPARATION OF WATER CHESTNUT-BASED BUTTERMILK (WCBM)

The water chestnut (*Trapa bispinosa*) powder was procured from local market; Lahore while milk was obtained from the Dairy Animals and Training and Research Center of University of Veterinary and Animal Sciences, Lahore. The cream was separated from the milk and cultured with *Streptococcus thermophillus* and *Lactobacillus bulgaricus*. The resulting ripened cream was churned, drained, and washed to produce butter and buttermilk as a by-product (Ewe & Loo 2016). Buttermilk was mixed with varying concentrations of water chestnut powder for each treatment, with WCB0 having no water chestnut, WCB1, WCB2, WCB3 and WCB4 containing 0.5%, 1%, 1.5%, and 2% (w/v) water chestnut powder, respectively. The buttermilk samples were packaged, pasteurized (65 **°C for 30 min)**, and stored at 4 °C for a month-long shelf-life study. Over the storage period, the samples were assessed for physicochemical, antioxidant, microbiological and sensory characteristics at 0, 7, 14, 21, and 28 days.

PROXIMATE AND YEAST AND MOLD ANALYSIS OF WATER CHESTNUT POWDER (WCP)

Proximate analysis (carbohydrates, proteins, fats, minerals and moisture contents) and yeast and mold analysis of WCP were performed according to the method described in Consumi et al. (2022).

PHYSICOCHEMICAL ANALYSIS OF BUTTER MILK

Buttermilk was analyzed for its physiochemical (fat%, moisture%, total solids%, protein%, pH, and acidity %) using standard methods defined in AOAC (2019).

YEAST AND MOLD COUNT

For the estimation of yeast and mold in buttermilk samples during storage period, 1 mL of each sample were analyzed by using spread platemethod on potato dextrose agar. Plates were incubated at 25-30 °C for 3-5 days. Yeast and mold will grow on the surface of the agar as visible colonies. Results were calculated after 3-5 days using colony counter (Banjara, Suhr & Hallen-Adams 2015).

ANTIFUNGAL ACTIVITY ASSAYS OF BUTTERMILK BY THE QUALITATIVE METHOD

The antifungal activity was evaluated as described in Dopazo et al. (2022) with a few modifications. Initially, the water chestnut-based buttermilk (WCBM) was frozen, lyophilised and suspended to a 400 g/L concentration with sterile distilled water. Then, Potato Dextrose Agar plates were sown with fungal spores using sterile swabs, and wells were punched in the agar with sterile 1000-µL pipette tips. A 100 µL of the WCBM was added to the wells, and plates were incubated for 48 h at 25 °C. After the incubation, the inhibition halos around each well were measured and marked as '' when no inhibition was observed, '+' when inhibition was from 1 to 4 mm, '++' when inhibition was from 4 to 10 mm and '+++' when inhibition was greater than 10 mm. Whey at the same concentrations was used as control.

FREE FATTY ACID ANALYSIS OF BUTTERMILK

For analysis of free fatty acid, 50 mL of ethanol was taken and few drops of phenolphthalein and 1% indicator were added and titrated against 0.1N NaOH until light pink colour appeared. Ten mL sample was added into the ethanol flask. It was heated for 2-3 min. If the pink colour persists after 2 min it means there are no free fatty acids. If the pink colour disappears it means free fatty acids are present. After the disappearance of colour, the flask containing buttermilk sample and ethanol was titrated against 0.1N NaOH. The final volume of 0.1N NaOH used was noted and free fatty acid% can be calculated by using this formula: (AOCS 1989).

$$\text{Free fatty acid \%} = \text{Volume used} \times 282 \times 100/1000 \times \text{weight of sample}$$

TOTAL ANTIOXIDANT CAPACITY (TAC)

The total antioxidant capacity was evaluated by phosphomolybdenum method as described by Nabasree and Bratati (2007). For the measurement of total antioxidant capacity (TAC), the reagent solution having 28 mM sodium phosphate, 4 mM ammonium molybdate, and 0.6 M sulfuric acid was taken. The sample (100 µL) was reacted with 1 mL reagent solution and heated to 95 °C for 90 min followed by cooling to 25 °C and measurements were conducted using spectrophotometer (double beam, Shimadzu, Japan) at 695 nm, and ascorbic acid was used as standard. The antioxidant capacities of samples were expressed as mg Ascorbic Acid Equivalent per gram of sample (mg AAE g).

TEXTURAL ANALYSIS OF WATER CHESTNUT BASED BUTTERMILK

PHASE SEPARATION OF BUTTERMILK

For phase separation of buttermilk, clear zone of samples was measured. Buttermilk samples were packed in glass bottles and can be easily seen inside out, so the clear zone was measured during 1^{st}, 7^{th}, 14^{th}, 21^{st}, 28^{th} days of storage (Quasem et al. 2009).

VISCOSITY

The viscosity of all samples was measured at 20 °C using digital viscometer (Brookfield model no DV2TLVTJ0) with A 61 probe at 100 rpm as described by AOAC (2016).

SENSORY ANALYSIS

Sensory analysis (appearance, phase separation, color, taste, and flavor) of buttermilk samples was based on intensity scale according to method described by Meilgaard, Civille and Carr (2007).

STATISTICAL ANALYSIS

Every treatment was run in five times, an experiment was planned in a Completely Randomized Design (CRD) and data were analyzed by two-way analysis of variance technique. Significant difference among the treatments was determined by Duncan Multiple Range Test using SAS 9.1 software (Steel, Torrie & Dicky 1997).

RESULTS AND DISCUSSIONS

PROXIMATE AND YEAST AND MOLD COUNT OF WATER CHESTNUT POWDER (WCP)

The proximate composition of 100 g of water chestnut powder was analyzed. The results indicated that carbohydrates are the primary macronutrient in *Trapa bispinosa*, comprising about 70% of its composition. Proteins account for 1.8%, while fats make up a mere 0.9%. The ash content, which represents the mineral composition, amounts to 10.32%, and moisture makes up 7.32% (Alfasane, Khondker & Rahman 2011). Yeast and mold count in water chest powder was found too few to count.

PHYSICOCHEMICAL ANALYSIS OF BUTTERMILK

The physicochemical analysis results of buttermilk showed that there was a significant variation ($p<0.05$) in protein percentage among treatments, while no significant relationship was observed during the storage days. This increasing trend in protein percentage is attributed to the high protein content of water chestnut. However, there was no significant difference ($p>0.05$) in fat percentage among treatments and storage days was observed (Table 1). The ash content results of water chestnut containing buttermilk indicated that the concentration of water chestnut powder had a significant impact ($p<0.05$) on the ash percentage of buttermilk. Additionally, the pH of buttermilk increased while the acidity decreased with the increase of water chestnut powder concentration. However, the pH decreased and acidity increased with the passage of the storage period. This phenomenon is due to the alkaline nature of water chestnut, which induced a difference among treatments (Peng & Jiang 2004). Furthermore, treatments had a significant effect ($p<0.05$) while storage period had non-significant effect on the moisture percentage of buttermilk.

YEAST AND MOLD COUNT OF WATER CHESTNUT POWDER-BASED BUTTERMILK

Results of yeast and mold indicated that treatments and storage had significant ($p<0.05$) effect on the growth of yeast and mold (Table 2). Mean values indicated that in all

TABLE 1. Compositional analysis of water chestnut buttermilk (WCBM)

	Storage Days					
	Treatments	0-days	07-days	14-days	21-days	28-days
Fat%	Control	0.80±0.01^{e}	0.81±0.05^{e}	0.80±0.09^{e}	0.82±0.02^{e}	0.80±0.02^{e}
	WCBM1	0.86±0.05^{d}	0.85±0.01^{d}	0.85±0.05^{d}	0.84±0.01^{d}	0.86±0.01^{d}
	WCBM2	0.91±0.02^{c}	0.90±0.02^{c}	0.91±0.01^{c}	0.92±0.03^{c}	0.91±0.03^{c}
	WCBM3	0.96±0.01^{b}	0.96±0.05^{b}	0.95±0.01^{b}	0.96±0.05^{b}	0.96±0.02^{b}
	WCBM4	1.05±0.03^{a}	1.04±0.01^{a}	1.05±0.03^{a}	1.01±0.05^{a}	1.01±0.05^{a}
Acidity%	Control	0.90±0.11^{a5}	0.95±0.41^{a4}	1.05.±0.20^{a3}	1.11±0.10^{a2}	1.17±0.22^{a1}
	WCBM1	0.86±0.22^{b5}	0.89±0.11^{b4}	0.98±0.29^{b3}	1.01±0.16^{b2}	1.05±0.07^{b2}
	WCBM2	0.80±0.07^{c5}	0.88±0.04^{d4}	0.92±0.33^{c3}	0.93±0.27^{b2}	0.98±0.12^{a1}
	WCBM3	0.75±0.10^{d5}	0.83±0.05 ^{d4}	0.87±0.11 ^{c3}	0.86±0.01^{b2}	0.92±0.01 ^{a1}
	WCBM4	0.70±0.23^{e5}	0.78±0.21^{e4}	0.81±0.21^{ab}	0.85±0.11^{b}	0.90±0.07^{ab}
Moisture%	Control	90.20±1.41^{a}	90.2±1.38^{a}	90.3±2.30^{a}	90.3±1.83^{a}	90.2±1.81^{a}
	WCBM1	90.16±1.53^{b}	90.14±1.45^{b}	90.14±1.72^{b}	90.15±1.38^{b}	90.15±1.44^{a}
	WCBM2	90.05±1.20^{c}	90.09±1.21^{c}	90.07±1.24^{c}	90.04±1.71^{c}	90.04±1.69^{b}
	WCBM3	89.99±1.15^{d}	89.22±1.21^{d}	89.3±1.95^{d}	89.2±1.44^{d}	89.2±1.40^{c}
	WCBM4	89.95±1.21^{e}	89.71±1.28^{e}	89.27±2.05^{e}	89.91±1.61^{e}	89.80±1.12^{d}
Protein %	Control	5.20±0.02^{e}	5.18±0.01^{d}	5.19±0.01^{e}	5.17±0.02^{e}	5.18±0.01^{e}
	WCBM1	5.46±0.07^{d}	5.47±0.03^{d}	5.45±0.05^{d}	5.47±0.01^{d}	5.48±0.04^{d}
	WCBM2	5.78±0.01^{c}	5.79±0.01^{c}	5.76±0.01^{c}	5.74±0.06^{c}	5.75±0.01^{c}
	WCBM3	6.09±0.06^{b}	6.08±0.05^{b}	6.08±0.07^{b}	6.07±0.01^{b}	6.05±0.05^{b}
	WCBM4	6.39±0.08^{a}	6.37±0.01^{a}	6.35±0.01^{a}	6.34±0.05^{a}	6.34±0.05^{a}

Values in the column and rows with different superscript letters and numbers are significantly different ($P<0.05$); Control: Buttermilk without addition of water chestnut powder (Control); WCBM1: Buttermilk with addition of water chestnut powder (0.5%); WCBM2: Buttermilk with addition of water chestnut powder (1.0%); WCBM3: Buttermilk with addition of water chestnut powder (1.5%); WCBM4: Buttermilk with addition of water chestnut powder (2.0%)

treatments colonies at 0 day of storage period were found too few too count. However, colonies increased in all treatments at 07 days of storage period. The yeast and mold count remained acceptable in WCBM3 and WCBM4 up to 14 days and at 21 days of storage period. Mean values of yeast and mold count in WCBM3 and WCBM4 at 21 days of storage period were 50±0.60 and 35±0.36, respectively. Inhibition of the growth of yeast and mold was noticed with the increase in water chestnut powder concentration. It is due to the presence of certain polyphenols in water chestnuts such as catechins and saponins that inhibit the growth of fungi (Romani et al. 2021). Moreover, water chestnuts also have peptides that have antifungal activity (Mandal et al. 2011).

TABLE 2. Yeast and mold

Parameters		Storage days			
Treatments	0-days	07-days	14-days	21-days	28 days
Control	TFTC	$60±0.61^{a4}$	$90±0.17^{a3}$	$130±0.26^{a2}$	$380±0.25^{a1}$
WCBM1	TFTC	$40±0.13^{b4}$	$65±0.15^{b3}$	$100±0.16^{b2}$	$340±0.15^{b1}$
WCBM2	TFTC	$30±0.25^{c4}$	$45±0.13^{c3}$	$90±0.12^{c2}$	$180±0.18^{c1}$
WCBM3	TFTC	$20±0.26^{d4}$	$30±0.58^{d3}$	$50±0.60^{d2}$	$100±0.97^{d1}$
WCBM4	TFTC	$15±0.15^{e4}$	$20±0.41^{e3}$	$35±0.36^{e2}$	$70±0.61^{e1}$

Values in the column and rows with different superscript letters and numbers are significantly different (P<0.05); Control: Buttermilk without addition of water chestnut powder (Control); WCBM1: Buttermilk with addition of water chestnut powder (0.5%); WCBM2: Buttermilk with addition of water chestnut powder (1.0%); WCBM3: Buttermilk with addition of water chestnut powder (1.5%); WCBM4: Buttermilk with addition of water chestnut powder (2.0%)

ANTIFUNGAL POTENTIAL OF WATER CHESTNUT POWDER-BASED BUTTERMILK

Results of antifungal potential of water chestnut-based buttermilk showed that treatments had significant (p<0.05) effect while storage period had non-significant effect on the growth inhibition of molds (Table 3). Mean values indicated that in control, WCBM1 and WCBM2 treatments inhibition were observed between 1-4 mm. Interestingly, in control treatment inhibition was close to WCBM1 and WCBM2, it might be due to the acidic environment that inhibits the growth of molds in control sample also. However, the inhibition level increased as the level of water chestnut powder concentration increased in WCBM3 and WCBM4. Inhibition in mold growth with the increase in water chestnut powder concentration might be due to the presence of saponins that disrupt the cell membrane of fungal cells, leading to their death. It may also be due to presence of chitinase enzyme that can break down the chitin, a component of fungal cell walls (Zhang et al. 2019).

ANTIOXIDANT POTENTIAL OF WATER CHESTNUT POWDER BASED BUTTERMILK

TOTAL ANTIOXIDANT CAPACITY (TAC)

Results of TAC showed that treatments and storage days had significant effect on inhibition of oxidation reactions (p<0.05) (Table 4). Antioxidant potential increased with the increased in WCP concentration in the buttermilk. However, as the storage days proceeded, TAC values decreased in all treatments. Mean values of TAC in WCBM3 and WCBM4 were 0.57±0.12 and 0.60±0.32, respectively, as compared to control 0.48±0.07 on the 21st day of storage. It is due to the presence of bioactive components such as flavonoids and polyphenols in the water chestnut that has antioxidant properties (Yu, Nanguet & Beta 2013). Shafi et al. (2016) also observed less oxidation when water chestnut powder (WCP) was used in baking products. In another study, water chestnut peel extracts reduced oxidation when used for cooking and frying of meat (Echegaray et al 2020).

FREE FATTY ACID PERCENTAGE

Mean values regarding free fatty acid% in water chestnut-based buttermilk indicated that treatments and storage significantly (p<0.05) effect the free fatty acid percentage of buttermilk. Mean value of free fatty acid% for control and WCBM4 were 0.87±0.12 and 0.79±0.07a at 0 day of storage. However, it was increased to 3.52±0.07 and 3.08±0.01 at 28 days of storage period for control and WCBM4, respectively. Free fatty acids percentage decreased with increase in water chestnut concentration (p<0.05). It is due to neutralization of free radicals by the antioxidants (flavonoids and polyphenols) present in the water chestnut powder (Zhan et al. 2016). You et al. (2007) studied that Chinese water chestnut extract, ascorbic acid and butylated hydroxytoluene were used as an antioxidant in food to prevent the linoleic acid oxidation and it was observed that food which was treated with water chestnut extract has more resistant against oxidation as compared to ascorbic acid and butylated hydroxytoluene.

TABLE 3. Antifungal potential of water chestnut powder-based buttermilk

Fungi	Control	WCBM1	WCBM2	WCBM3	WCBM4
Penicillium camemberti	+	+	+	++	++
Penicillium expansum	+	+	+	++	++
Penicillium roqueforti	+	+	+	++	++
Penicillium digitatum	+	+	+	++	++
Penicillium brevicopactum	+	+	+	++	++

(+) indicated the inhibition of fungus between 1-4 mm; (++) indicated the inhibition of fungus >4 mm. Control: Buttermilk without addition of water chestnut powder (Control); WCBM1: Buttermilk with addition of water chestnut powder (0.5%); WCBM2: Buttermilk with addition of water chestnut powder (1.0%); WCBM3: Buttermilk with addition of water chestnut powder (1.5%); WCBM4: Buttermilk with addition of water chestnut powder (2.0%)

TABLE 4. Effect of water chestnut on total antioxidant capacity of buttermilk during storage

Parameters	Storage days				
Treatments	0-days	07-days	14-days	21-days	28 days
Control	0.66 ± 0.07^{e1}	0.59 ± 0.31^{e2}	0.51 ± 0.05^{e3}	0.48 ± 0.07^{e4}	0.42 ± 0.12^{e5}
WCBM1	0.67 ± 0.05^{d1}	0.60 ± 0.17^{d2}	0.56 ± 0.11^{d3}	0.51 ± 0.10^{d4}	0.45 ± 0.01^{d5}
WCBM2	0.69 ± 0.10^{c1}	0.62 ± 0.05^{c2}	0.58 ± 0.12^{c3}	0.53 ± 0.07^{c4}	0.48 ± 0.11^{c5}
WCBM3	0.71 ± 0.07^{b1}	0.67 ± 0.31^{b2}	0.61 ± 0.05^{b3}	0.57 ± 0.12^{b4}	0.54 ± 0.13^{b5}
WCBM4	0.76 ± 0.11^{a1}	0.69 ± 0.07^{a2}	0.63 ± 0.10^{a3}	0.60 ± 0.32^{a4}	0.59 ± 0.11^{a5}

Values in the column and rows with different superscript letters and numbers are significantly different ($p<0.05$)

TEXTURE ANALYSIS

Mean values (Table 6) showed that treatments and storage days had a significant ($p<0.05$) effect on the phase separation and viscosity of water chestnut-based buttermilk. Water chestnut treated samples had significantly lower phase separation and high viscosity as compared to control. The mean values for phase separation and viscosity of control and WCBM4 samples were observed 1.8±0.11 and 1.50±0.07, respectively, at 0 day of storage. Phase separation decreased with an increase in water chestnut concentration due to its high fiber and starch content that has the ability to form a gel-like network and reduce phase separation in buttermilk (Latoch, Libera & Stasiak 2019). Phase separation increased while viscosity decreased in control and water chest nut treated samples with the passage of storage. During storage, increase in phase separation and reduction in viscosity is due to increase in acidity that denatures the emulsifying agents and emulsions gets destabilize (Tesch & Schubert 2002).

CONCLUSION

Overall, the findings of this study highlight the potential of water chestnut as an ingredient to improve the

TABLE 5. Effect of water chestnut on free fatty acids % of buttermilk during storage

Parameters	Storage days				
Treatments	0-day	07-days	14-days	21-days	28 days
Control	0.87±0.12^{a1}	1.28±0.10^{a1}	1.95±0.11^{a1}	2.91±0.01^{a1}	3.52±0.07^{a1}
WCBM1	0.85±0.05^{b2}	1.18±0.05^{b2}	1.56±0.10^{b2}	2.53±0.10^{b2}	3.36±0.11^{b2}
WCBM2	0.83±0.07^{c3}	1.09±0.10^{c3}	1.48±0.01^{c3}	2.46±0.11^{c3}	3.29±0.05^{c3}
WCBM3	0.80±0.10^{d4}	1.01±0.01^{d4}	1.36±0.07^{d4}	2.37±0.01^{d4}	3.13±0.01^{d4}

Values in the column and rows with different superscript letters and numbers are significantly different ($p<0.05$)

TABLE 6. Effect of water chestnut on phase separation of buttermilk during storage

Parameters	Storage days				
Treatments	0-day	07-days	14-days	21-days	28 days
Control	1.8±0.11^{a5}	1.72±0.05^{a4}	2.5±0.21^{a3}	2.8±0.07^{a2}	3.5±0.18^{a1}
WCBM1	1.89±0.13^{b5}	1.53±0.20^{b4}	2.1±0.11^{b3}	2.3±0.10^{b2}	3.1±0.22^{b1}
WCBM2	1.70±0.05^{c5}	1.42±0.13^{c4}	1.73±0.10^{c3}	2.1±0.13^{c2}	2.5±0.11^{c1}
WCBM3	1.60±0.10^{d5}	1.31±0.32^{d4}	1.62±0.04^{d3}	1.89±0.11^{d2}	2.32±0.09^{d1}
WCBM4	1.50±0.07^{e5}	1.24±0.28^{e4}	1.51±0.20^{e3}	1.78±0.06^{e2}	2.18±0.08^{e1}

Values in the columns and rows with different superscript letters and numbers are significantly different ($p<0.05$)

TABLE 7. Effect of water chestnut on viscosity of buttermilk during storage

Parameters	Storage days				
Treatments	0-day	07-days	14-days	21-days	28 days
Control	0.73±0.22^{a1}	0.65±0.10^{b2}	0.53±0.20^{c3}	0.48±0.41^{c4}	0.43±0.11^{d5}
WCBM1	0.83±0.07^{a1}	0.76±0.16^{b2}	0.65±0.09^{c3}	0.59±0.11^{d4}	0.56±0.02^{d5}
WCBM2	1.20±0.12^{a1}	1.18±0.07^{b2}	1.13±0.03^{c3}	1.09±0.04^{c4}	1.03±0.01^{d4}
WCBM3	1.81±0.01^{a1}	1.78±0.01^{b2}	1.61±0.11^{c3}	1.59±0.05^{d4}	1.55±0.01^{d5}
WCBM4	2.22±0.07^{a1}	2.19±0.11^{b2}	2.14±0.21^{c3}	2.08±0.21^{d4}	2.05±0.03^{e5}

Values in the columns and rows with different superscript letters and numbers are significantly different ($p<0.05$)

physicochemical properties, antioxidant activity, antifungal potential, and textural stability of buttermilk. These positive outcomes offer promising opportunities for the commercial utilization of buttermilk in the dairy industry, addressing the challenges associated with its limited shelf life and susceptibility to fungal growth. The incorporation of WCP could lead to the development of value-added buttermilk products that appeal to consumers and provide economic benefits to the dairy industry. Further research and development in this area are warranted to explore the full potential of water chestnut as an ingredient in buttermilk and to optimize the formulation for commercial applications.

ACKNOWLEDGMENTS

The authors express their gratitude to the Department of Dairy Technology, University of Veterinary and Animal Sciences, Lahore for providing research support and facilities.

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*Corresponding author; email: nabila.gulzar@uvas.edu.