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RESEARCH ARTICLE

EFFECT OF ADDITION OF *Withania somnifera* (ASHWAGANDHA) ROOT POWDER ON SELECTED PROPERTIES OF COW MILK

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Abstract

Withania somnifera **(Ashwagandha) is an important tropical herb with proven health benefits which has an enormous potential to be used in functional food applications. The present study aimed to find out the effect of the addition of *W. somnifera* root powder (WSRP) on the compositional, physicochemical, physical, functional and sensory properties of cow milk. Skimmed and standardized (3% fat) milk were separately fortified with WSRP at the rate of 0, 0.5, 1.0, 1.5 and 2 percent levels, well mixed and pasteurized (63°C for 30 min) ending up in 10 treatments. 2 x 5 (milk type x WSRP level) factorial arrangement of treatments in a completely randomized design was used with 3 replicates and SPSS (ver. 20) was used for the data analysis. Significant (p<0.05) reduction of rennet coagulation time (RCT) was observed with increasing WSRP percentage irrespective of the type of milk. Standardized milk had significantly (p<0.05) lower RCT than skimmed milk. Instrumental colour values were significantly (p<0.05) affected by the added WSRP in both milk types while fermentation characteristics were not affected. Sensory scores were decreased with increasing levels of WSRP in both milk types due mainly to the sedimentation effect. From the above results, it is concluded that a possibility exists to fortify milk with WSRP and produce functional fermented dairy products such as cheese and yoghurt. Nevertheless, studies are needed with other forms of *W. somnifera* such as root powder extract to reduce the impact of sedimentation.**

Keywords: Functional dairy products, Phytochemicals, Rennet coagulation time, *Withania somnifera*

INTRODUCTION

Around 71% of global deaths have been reported due to non-communicable diseases (NCDs) such as cardiovascular diseases, cancer, chronic respiratory diseases, diabetes etc. which kill around 41 million people each year (WHO 2021). In Sri Lanka NCDs are estimated to account for nearly 83% of all deaths (WHO 2018). Unacceptable dietary habits are one of the leading causes of this among others such as personal behaviours, environmental elements, busy and stressful lifestyles and some hereditary characteristics (Ezzati and Riboli 2013). Nevertheless, due to the increased awareness of the relationship between health and diet, people have moved to a new trend of dieting and are willing to consume foods that offer health benefits than luxury foods. Therefore, there is a huge demand for functional foods in the global food market.

Even though there is no globally accepted definition for functional foods, in broad terms they can be described as foods that provide

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health benefits beyond basic nutrition (Bradford 2015). It is evident that functional foods can effectively address the aforementioned health-related challenges, whilst satisfying hunger and providing people with necessary nutrients. Functional foods can be developed in many ways and one such approach is by incorporating conventional foods with plant ingredients which are having medicinal value. Plants are a rich source of biologically active substances called phytochemicals. Phytochemicals are a large group of plant-derived compounds that are responsible for disease protection (Veena *et al*. 2015) and are responsible to improve the digestion, cardiovascular activity and emotional state of individuals (Sukhikh *et al.* 2019).

Ashwagandha (*Withania somnifera*) is an important medicinal plant belongs to the family Solanaceae which has been used in traditional medicinal systems like Ayurveda, Unani and Siddha for its therapeutic benefits for more than 5000 years (Mandlik Ingawale and Namdeo 2021). It is considered a vital herbal restorative in Ayurvedic medicine which can be used for various pharmacological properties. It has been reported that *W. somnifera* possess many beneficial health properties such as antioxidant (Chaurasia *et al*. 2000), antimicrobial including antiviral (Pant *et al*. 2012), immunomodulatory (El-Boshy *et al*. 2013), anti-inflammatory (Chandra *et al*. 2012), anticancer (Wadhwa *et al*. 2013), adaptogenic, cardioprotective (Dhuley 2000) etc. The plant is rich in phytochemical compounds such as steroidal lactones (withanolides, withaferins), steroidal alkaloids, saponins, flavonoids, phenols, carbohydrates, glycosides, phytosterols, terpenoids etc. (Tiwari *et al*. 2014; Swaminathan and Santhi 2019) and are responsible for those proven health benefits. Singh *et al*. (1982) reported that according to animal toxicity studies, *W. somnifera* and its constituents are safe even when administered in high doses.

Milk is one of the most widely consumed nutritious foods in the world and an effective carrier that can be used to deliver phytochemicals which give beneficial health effects by developing into functional dairy products. Sawale *et al.* (2012) mentioned that the lipid-soluble components and water-soluble active constituents of herbs may be easily assimilated in milk fat and the water in milk, respectively. However, the addition of herbs, their powders or extracts into milk and subsequent processing conditions poses a challenge since there is a possibility for having interactions among the components of the milk and the herb (Sawale *et al*. 2012). Such interactions may lead to practical difficulties in developing functional dairy products and therefore, it is of utmost importance to study the effect of the addition of these plant materials on the properties of such food systems before being developed into a marketable product. Within this context, the present study was carried out to find out the effect of the addition of *W. somnifera* root powder (WSRP) on the physical, physicochemical, functional and sensory properties of cow milk.

MATERIALS AND METHODS

Fresh cow milk was obtained from the milking herd maintained at the farm of the Faculty of Agriculture, University of Ruhuna, Sri Lanka. Freeze-dried thermophilic yoghurt culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (1:1 ratio) and microbial rennet (CHY-MAX Extra) were obtained from Chr. Hansens Laboratories, Denmark. Commercially available 100% natural WSRP was obtained from a well-recognized Ayurvedic drug store located in Kandy, Sri Lanka. All the chemicals used were of analytical grade.

Preparation of milk samples fortified with WSRP

Raw cow milk was preheated (40ºC) and cream separated to obtain cream and skimmed milk. Part of the skimmed milk was standardized to 3% fat by adding a calculated amount of cream according to Pearson's square method. Skimmed and 3% fat standardized milk were separately fortified with WSRP at the rate of 0, 0.5, 1.0, 1.5 and 2 percent levels (w/w) ending up in 10 treatments. The milk samples were separately mixed using a glass rod and pasteurized at 63°C for 30 minutes and refrigerated (4±1°C) before being used for the analysis.

Analysis of WSRP fortified milk

pH, titratable acidity and specific gravity

pH of control and experimental milk samples was measured by using a digital pH meter (HANNA HI9811-5) after calibrating with fresh pH 4, 7 and 9 standard buffer solutions at 25°C. Titratable acidity was determined by titrating the milk samples with 0.1 N NaOH using 0.5% phenolphthalein indicator. The specific gravity of milk samples was determined by using a lactometer.

Proximate composition

The total solids (TS) content of control and experimental milk samples was determined by gravimetric method. Gerber method was used to determine the fat content. Protein content was determined by the micro-Kjeldahl method. The Ash content of milk samples was determined by the ignition method (AOAC 2000). The Carbohydrate (CHO) content of the milk samples was calculated by subtracting protein, fat and ash percentages from the percentage of the total solids.

Rennet coagulation time (RCT)

Rennet coagulation time of control, WSRP fortified skimmed and standardized milk was determined according to the method proposed by Wheelock (1971). Briefly, 0.2 ml of 1% solution of microbial rennet (CHY-MAX Extra) was added to 5 ml milk in a test tube which has equilibrated at 37ºC. A stopwatch was started at the time of addition of rennet solution and the content in the tube was gently rotated while kept in a water bath maintained at 37ºC. Rennet coagulation time was taken as the time required for appearing a layer of film of milk inside the wall of the test tube.

Fermentation characteristics

Control, WSRP added skimmed and standardized milk samples were subjected to microbial fermentation to determine the changes in pH and titratable acidity during the incubation of 3 h at 42±1ºC. Thermophilic yoghurt starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (1:1 ratio) was used to inoculate the milk samples and the measurements were taken at 30 min intervals during incubation.

Instrumental Colour

Instrumental colour analysis of control, WSRP fortified skimmed and standardized milk samples were performed by using a PCE-CSM 2 colourimeter after calibrating with the white calibration plate provided by the manufacturer and as specified. CIELab colour scale was used to get L^*, a^* and b^* values. The L^* value ranges from 0 to 100, indicates the brightness from black to white; the a^* value indicates the variation from red (+a^*) to green (-a^*); the b^* value indicates the variation from yellow (+b^*) to blue (-b^*) in the CIELab colour scale.

Sensory evaluation

Pasteurized control, WSRP fortified skimmed and standardized milk samples were evaluated for their sensory characteristics by offering to a 30-qualified panel of judges. Non-smoking healthy adults who were willing to participate in the study were selected from the staff and the student community of the Faculty of Agriculture, University of Ruhuna, Sri Lanka for the sensory evaluation. Individual sensory booths were prepared with the samples, drinking water to wash off the mouth and a sensory evaluation sheet. The place was well ventilated and ample lighting was provided to eliminate disturbances from nuisance odours and insufficient light conditions, respectively. Each sample was labelled with random three-digits and the order of presentation of samples was randomized for each panellist. They were requested to evaluate the samples quickly but not in a hurry for colour and appearance, smell, taste, mouth feel, after taste, sedimentation and overall acceptability using a 5-point hedonic scale (1= dislike extremely, 2=dislike slightly, 3=neither like nor dislike, 4=like slightly and 5= like extremely).

Statistical Analysis

A two-factor (type of milk and level of WSRP: 2×5) factorial arrangement of treatments in a completely randomized design was used as the statistical design with 3 replicates. Parametric data were analyzed by analysis of variance (ANOVA) procedure using the SPSS (ver. 21) statistical software package. The results were expressed as mean±SD. Sensory data were analyzed by using Friedman non-parametric test.

RESULTS AND DISCUSSION

Proximate composition of WSRP fortified milk

The proximate composition provides important information about the medicinal and nutritional value of a product. Table 1 shows the proximate composition of skimmed and standardized (3% fat) milk fortified with WSRP.

It was observed that irrespective of the type of milk, TS and CHO percentages had an increasing trend with the increasing level of WSRP addition, even though, a slight exception was observed in the CHO percentage in standardized milk at 2% WSRP level (Table 1). Based on the statistical analysis, there was no interaction effect (F (4, 20)=0.274, p=0.891) between the milk type and the WSRP level on TS value. However, it was observed that the main effect of milk type on TS value was significant (F (1, 20) =47.371, p=0.000) due mainly to the obvious reason of having milk fat in standardized compared to skim milk. Further, the main effect of WSRP level on TS value was also significant such that increasing the WSRP level in milk, the TS value increased (F (4, 20)=3.490, p=0.026). Also, it was observed that there was no interaction effect between the milk type and the WSRP level on CHO value (F (4, 20)=0.790, p=0.545). The main effect of milk type on CHO value was also not significant (F (1, 20)=0.22, p=0.883). However, the main effect of WSRP level on CHO value was significant such that with increasing the WSRP level in milk, the CHO value increased (F (4, 20)=7.206, p=0.001). It has been reported that WS root powder contains around 92.55% TS of which 50% is CHOs and 32.3% is crude fibre (Kumari and Gupta 2016) and proved the reason for the above observations. Fat, protein and ash percentages did not show any significant (p>0.05) difference in both milk types with the increased level of WSRP (Table 1).

pH and titratable acidity of WSRP fortified milk

Table 2 depicts the effect of fortification of WSRP on the physicochemical and physical properties of skimmed and standardized cow milk. Even though a slight reduction was observed in the pH with the increase of WSRP level in milk regardless of the type,

Table 1: Mean ± SD of compositional parameters of milk fortified with WSRP

Milk Type	WSRP Level (%)	Composition (%)				
		TS	Fat	Protein	CHO	Ash
SM	0.0	7.73^{a}±0.80	0.10^{a}±0.00	3.44±1.01	3.68^{a}±0.39	0.65±0.14
	0.5	8.59^{ab}±1.68	0.10^{a}±0.00	2.80±1.01	5.15^{abc}±1.35	0.56±0.14
	1.0	9.14^{ab}±1.20	0.10^{a}±0.00	3.22±1.11	5.23^{abc}±0.39	0.59±0.12
	1.5	9.55^{abc}±0.62	0.10^{a}±0.00	3.00±1.43	5.83^{abc}±0.78	0.62±0.15
	2.0	10.19^{abc}±1.08	0.10^{a}±0.00	2.59±1.42	6.95^{c}±0.83	0.54±0.08
SDM	0.0	10.73^{bc}±0.54	3.13^{b}±0.12	3.63±1.33	4.26^{ab}±1.28	0.48±0.03
	0.5	11.52^{bc}±0.59	3.10^{b}±0.10	2.68±0.87	4.94^{abc}±1.17	0.57±0.09
	1.0	11.28^{bc}±1.22	3.07^{b}±0.12	2.62±0.89	5.06^{abc}±0.76	0.54±0.01
	1.5	12.28^{c}±1.22	3.07^{b}±0.12	2.38±1.11	6.38^{bc}±0.74	0.57±0.05
	2.0	12.26^{c}±0.65	3.10^{b}±0.10	2.63±1.02	5.96^{abc}±0.66	0.57±0.05

$^{a, b, c}$ Means with different superscripts within each column differ significantly (p<0.05); SM: skim milk; SDM: standardized milk; TS: total solids; CHO: carbohydrate; WSRP: *W. somnifera* root powder significant differences were not observed ($p>0.05$) and ranged from 6.44±0.33 to 6.53±0.33 in skim milk and from 6.47±0.35 to 6.55±0.35 in standardized milk. Further according to the statistical analysis, significant interaction effects, as well as main effects, were not detected for the pH of WSRP fortified cow milk. Similar observations were detected by Veena *et al.* (2015) who reported that there was no significant change in the pH even though an apparent difference was observed, in milk fortified with Shatawari extract. Further, they suggested that this apparent difference in pH might be due to the presence of acidic components (ascorbic acid) in the herbal extract. *W. somnifera* also contains acidic compounds as reported by Saleem *et al.* (2020) and Mukherjee *et al.* (2020) which can influence the pH of the fortified milk.

With respect to TA, even though significant differences were not observed in each type of milk, there was a significant ($p<0.05$) difference between at 0 level of WSRP fortified skim milk and at 2% level of WSRP fortified 3% fat standardized milk. Further, it was observed that TA showed an increasing trend with the increment of WSRP in both types of milk and ranged from 0.170±0.012 to 0.196±0.005 amongst the skim milk and from 0.178±0.005 to 0.203±0.005 amongst the standardized milk (Table 2). Apart from that, it is clear that the 3% fat standardized milk fortified with WSRP sowed slightly higher TA values than their respective skimmed milk counterpart even though, significant differences were not observed. Veena *et al.* (2015) reported an apparent increment of TA, even though not significant, in the milk fortified with Shatawari extract. In the present study slight reduction of pH and increment in TA in milk with the increase of the level of WSRP might be due to the presence of various chemical compounds especially organic acids (Mukherjee *et al.* 2020; Saleem *et al.* 2020) in *W. somnifera* as mentioned previously.

Specific gravity of WSRP fortified milk

Statistical analysis showed that there was no interaction effect of milk type and WSRP level on SG of fortified milk (F (4, 20)=0.943, p=0.459). However, the increase in SG induced by the addition of WSRP was significant (F (4, 20)=11.962, p=0.000). This observation is obviously due to the presence of various compounds which were incorporated with the addition of WSRP into milk during the fortification. Furthermore, higher values of SG were observed in the skimmed milk compared to its respective 3% fat standardized counterpart and statistical analysis showed that it was significant (F (1, 20)=30.189, p=0.000). It is a well-known fact that the specific gravity of standardized milk is lower than the skimmed milk since the fat

Table 2: Mean ± SD of physicochemical and physical properties of milk fortified with WSRP

	WSRP Level (%)	Physicochemical Properties		Physical Properties	
		pH	TA (% LA)	SG	RCT (min)
SM	0.0	6.53±0.33	0.170^{a}±0.012	1.036^{abcd}±0.001	29.75^{g}±1.57
	0.5	6.52±0.33	0.176^{ab}±0.005	1.037^{bcd}±0.002	27.33^{g}±0.17
	1.0	6.48±0.28	0.182^{ab}±0.023	1.038^{cd}±0.001	23.71^{f}±0.37
	1.5	6.45±0.33	0.195^{ab}±0.002	1.039^{cd}±0.000	20.69^{e}±0.55
	2.0	6.44±0.33	0.196^{ab}±0.005	1.040^{d}±0.001	16.69^{d}±0.45
SDM	0.0	6.55±0.35	0.178^{ab}±0.005	1.0333^{ab}±0.002	12.82^{c}±1.59
	0.5	6.49±0.40	0.185^{ab}±0.008	1.0330^{a}±0.001	11.83^{c}±1.28
	1.0	6.49±0.40	0.190^{ab}±0.010	1.035^{abc}±0.001	11.36^{bc}±0.96
	1.5	6.48±0.36	0.195^{ab}±0.008	1.037^{bcd}±0.002	8.84^{ab}±0.44
	2.0	6.47±0.35	0.203^{b}±0.005	1.038^{cd}±0.001	6.27^{a}±0.17

$^{a, b, c, d, e, f, g}$ means with different superscripts within each column differ significantly ($p<0.05$); SM: skim milk; SDM: standardized milk; TA: titratable acidity; LA: lactic acid; SG: specific gravity; RCT: rennet coagulation time; WSRP: *W. somnifera* root powder in the standardized milk is lighter and reduces its specific gravity

Rennet coagulation time of WSRP fortified milk

As depicted in Table 2, RCT was significantly ($p<0.05$) decreased by the addition of WSRP irrespective of the type of milk. This observation is very important in cheese manufacturing where the time taken for milk coagulation is of great concern in the reduction of the cost of production. This coagulation process continues faster with the formation of firm clots as the pH lowered below that of the milk. The reduction of pH affects the stability of the casein micelles by the release of the calcium ions (Veena *et al.* 2015) which facilitates the coagulation of milk. Ostersen *et al.* (1997) also reported that the RCT is primarily affected by the pH of the milk. As reported elsewhere in this study, the apparent reduction of milk pH due to the addition of WS root powder might be the reason for the observed results.

Further, it was observed that the RCT of standardized milk was significantly ($p<0.05$) lower compared to that of the respective skimmed counterpart. According to the results it is suspected that fat influences controlling RCT. As reported by Veena *et al.* (2015), fat influences the first steps of the union of the destabilized micelles. They further suggested that the possible changes in the fat globule membrane during the heat treatment could improve the destabilized micelles aggregation in whole milk. Further, the authors emphasized that the aggregation of denatured whey proteins to the fat globule membrane due to the application of heat may reduce the aggregation of these proteins with κ-casein, influencing directly the rennet coagulation action and time.

Instrumental colour of WSRP fortified milk

The Colour of food is an important parameter to be considered since it directly influences consumer preference (Chye *et al.* 2012). Table 3 shows the instrumental colour values detected in WSRP fortified skimmed and 3% fat standardized milk. It is clear from the table that the colour parameters were significantly affected by the addition of WSRP irrespective of the type of milk.

Whiteness or lightness in fluid milk results from the presence of colloidal particles, such as milk fat globules and casein micelles, capable of scattering light in the visible spectrum (Fox and McSweeney 1998). Further, Dufossé and Galaup (2021) reported that the milk constituents such as fat, protein, Ca, and P as well as processing conditions affect the physical structure of milk which in turn alter the L^* value. As shown in Table 3,

Table 3: Mean ± SD of instrumental colour parameters# of milk fortified with WSRP

Milk Type	WSRP Level (%)	Instrumental colour parameter		
		L^*	a^*	b^*
SM	0.0	$52.89^{c}\pm2.18$	$-3.16^{a}\pm0.12$	$0.97^{a}\pm0.07$
	0.5	$50.13^{bc}\pm3.73$	$-2.17^{b}\pm0.03$	$2.81^{b}\pm0.17$
	1.0	$55.97^{c}\pm1.53$	$-1.70^{b}\pm0.28$	$3.20^{bc}\pm0.42$
	1.5	$41.77^{ab}\pm3.78$	$-0.63^{c}\pm0.11$	$3.19^{bc}\pm1.19$
	2.0	$32.89^{a}\pm3.31$	$-0.09^{cd}\pm0.58$	$4.07^{cd}\pm0.36$
SDM	0.0	$47.57^{bc}\pm2.89$	$-1.46^{b}\pm0.28$	$4.57^{de}\pm0.10$
	0.5	$54.07^{c}\pm1.22$	$-0.61^{c}\pm0.03$	$5.40^{ef}\pm0.05$
	1.0	$42.42^{ab}\pm6.48$	$-0.22^{cd}\pm0.20$	$4.87^{de}\pm0.26$
	1.5	$40.13^{ab}\pm2.63$	$0.51^{de}\pm0.18$	$5.43^{ef}\pm0.09$
	2.0	$47.90^{bc}\pm3.97$	$0.66^{e}\pm0.29$	$6.35^{f}\pm0.06$

$^{a, b, c, d, e, f}$Means with different superscripts within each column differ significantly ($p<0.05$)
SM: skim milk; SDM: standardized milk; WSRP: *W. somnifera* root powder
$^{\#}L^*$: lightness, a^*: redness (+) and greenness (-), b^*: yellowness (+) and blueness (-)

the addition of WSRP into milk affected the lightness value significantly ($p<0.05$). It was observed that even with some exceptions (during the study it was noted that there was quick sedimentation of WSRP in milk which was difficult to control), a decreasing trend of the L^* value with the increasing level of WSRP in skimmed as well as in standardized milk. *W. somnifera* root powder used in the current study had off white to light brown colour. The contribution of light brown colour to milk from the added WSRP and Millard products during the heating process of milk might be responsible for the changes in the lightness value. Veena *at el*. (2015) observed a decrease in lightness in the milk fortified with Shatawari root powder extract. Moreover, Hashim *et al*. (2009) reported that the addition of date fibre significantly lower the L^* value of yoghurt. In addition to that, in the current experiment, it was observed that standardized milk had low L^* values with few exceptions compared to its skimmed counterpart. Pigments such as carotene in the fat fraction in standardized cow milk might be the reason for this observation.

Skimmed as well as standardized milk fortified with WSRP had negative a^* values except at 1.5 and 2% levels in the standardized milk, indicating the green tone over the red (Table 3). At 0 level of WSRP, i.e. in the control, significantly ($p<0.05$) lower a^* values were observed irrespective of the type of milk. With increasing the level of WSRP, a decrease of the green tone was observed. Therefore the redness of the milk depends on the level of WSRP in milk. The used WSRP in the present study was slightly off-white and this observation of changing towards red is acceptable. Similar observations were made in skimmed as well as in standardized milk. This is in line with the observations made by Veena *et al*. (2015) for milk fortified with shatawari root powder extract. All the b^* values observed in the current study were above zero indicating that the yellowness is dominating over the blue in WSRP fortified both skimmed as well as in standardized milk. Further with increasing the level of WSRP, b^* value was increased (Table 3). As mentioned elsewhere, off white colour of WSRP could be the reason for that. It is observed that the yellowness of the WSRP fortified standardized milk is significantly ($p<0.05$) higher than their skimmed milk counterpart. The yellowness of milk which is largely governed by the amount of β-carotene pigment present in the milk fat fraction (Agabriel *et al*. 2007; Alothman *et al*. 2019) might be the reason for that.

Sensory attributes of WSRP fortified milk

The market success of any food product depends on consumer acceptability. Therefore, along with the nutritional and other health-related aspects, maintaining sensory quality is crucial for a functional food product to be accepted by consumers (Narayana *et al*. 2020). Johanson *et al*. (2010) reported that nutritional information is independent of the strength of the sensory attributes. Moreover, Verbeke (2006) reported that the consumer response can be modified for a particular product if the sensorial characteristics are altered. Keeping the idea of future developments of functional dairy products fortified with *W. somnifera* that satisfy the consumers in mind, the experimental milk samples were offered to a sensory panel for their subjective sensory evaluation. According to the results, it is clear that the scores of all the sensory attributes, except smell in skimmed milk, were significantly ($p<0.05$) affected by the addition of WSRP (Table 4). With the increment of the WSRP level in milk, a reduction in sensory scores was noted and the control milk sample received the highest score for all the sensory attributes irrespective of the type of milk. Along with the harsh flavour, as reported by some members in the sensory panel, quick sedimentation of WSRP in milk samples which was observed during the experiment had a significant influence on sensory characteristics, even though in general, all the sensory scores were decreased. Overall acceptability score, comparable to that of the control sample was detected at the lowest level (0.5%) of WSRP fortification in skimmed as well as standardized milk. Landge *et al*. (2011) prepared shrikhand, an Indian dairy product by incorporating WSRP at the rate of 0.3%, 0.5% and 0.7% and concluded that the product made using 0.5% was superior with respect to organoleptic parameters. It would be worthwhile to further investigate the use of different forms of WS such as root powder extract, in the development of functional dairy-based products, so that optimum levels could be incorporated to obtain maximum health benefits.

Veena *et al*. (2015) in their study used a freeze-dried form of aqueous extract of shatawari root powder at the rate of 1% level in milk which was selected based on the preliminary sensory evaluation trials to examine the feasibility of developing future functional dairy products.

Fermentation characteristics of WSRP fortified milk

The changes in pH of WSRP fortified pasteurized skimmed and 3% fat standardized cow milk during the incubation period of 3 h at 42±1°C are illustrated in Fig. 1a and Fig. 1b, respectively.

Initial pH of yoghurt starter culture (*S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, 1:1 ratio) added WSRP fortified pasteurized skimmed milk samples varied between 6.42 (at 2% WSRP level) and 6. 50 (control) while in standardized milk samples the initial pH was observed in the range of 6.43 (at 2% WSRP level) to 6.52 (control). This shows that the addition of WSRP reduced the initial pH of the milk base even though, the differences were not significant (p>0.05). do Espirito Santo *et al.* (2012) reported that the probiotic yoghurt milk base added with passion fruit peel powder had a significantly lower pH compared to the control. Acidic compounds present in WS as

Table 4: Effect of fortification with WSRP on sensory attributes of milk

Milk Type	WSRP Level (%)	Sensory score[#]						
		Taste	Smell	Mouth feel	Colour and Appearance	After-taste	Sedimentation	Overall Acceptability
SM	0.0	4.06^{b} ±0.77	3.19± 0.98	3.68^{bc} ±0.95	4.42^{b} ±0.85	3.90^{b} ±0.83	4.29^{c} ± 0.90	4.00^{b} ±0.82
	0.5	2.94^{a} ±1.26	2.97± 0.91	3.10^{abc} ±1.27	3.97^{ab} ±1.02	2.94^{ab} ±1.15	3.52^{bc} ±1.21	3.26^{ab} ±1.18
	1.0	2.61^{a} ±0.96	2.81± 0.98	2.90^{ab} ±1.01	3.94^{ab} ±0.85	2.58^{a} ±1.09	2.61^{a} ± 1.20	2.81^{a} ±1.05
	1.5	2.52^{a} ±1.06	2.97± 0.95	2.52^{a} ±1.06	3.52^{a} ±1.06	2.26^{a} ±1.15	2.77^{ab} ±1.20	2.55^{a} ±1.03
	2.0	2.55^{a} ±1.21	2.81± 1.11	2.68^{a}± 1.40	3.39^{a} ±1.26	2.45^{a} ±1.29	2.61^{a} ± 1.28	2.61^{a} ±1.36
SDM	0.0	4.55^{c} ±0.89	4.32^{c} ±1.11	4.42^{c} ±1.06	4.58^{b} ±0.85	4.55^{b} ±0.93	4.32^{c} ± 1.17	4.68^{c} ±0.83
	0.5	3.39^{b} ±1.02	3.65^{bc} ±0.92	3.39^{bc} ±1.02	4.23^{ab} ±0.96	3.55^{b} ±1.06	3.42^{bc} ±1.21	3.61^{bc} ±0.99
	1.0	2.61^{ab} ±0.92	3.42^{b} ±0.89	2.68^{ab} ±0.98	3.77^{a} ±0.88	2.48^{a} ±0.81	2.58^{ab} ±1.18	2.71^{ab} ±1.07
	1.5	2.29^{a} ±0.86	3.23^{ab} ±1.12	2.32^{a} ±1.01	3.58^{a} ±1.03	2.16^{a} ±0.86	2.45^{ab} ±1.12	2.29^{a} ±0.90
	2.0	2.00^{a} ±1.12	2.61^{a} ±1.12	2.13^{a} ±1.15	3.26^{a} ±1.37	2.13^{a} ±1.12	1.84^{a} ± 1.24	2.00^{a} ±1.13

[a, b, c]Mean±SD with different superscripts within each column and within each milk type differ significantly (p<0.05); SM: Skimmed milk; SDM: Standardized milk; WSRP: *W. somnifera* root powder
[#]5-point hedonic scale was used where 1= dislike extremely and 5= like extremely

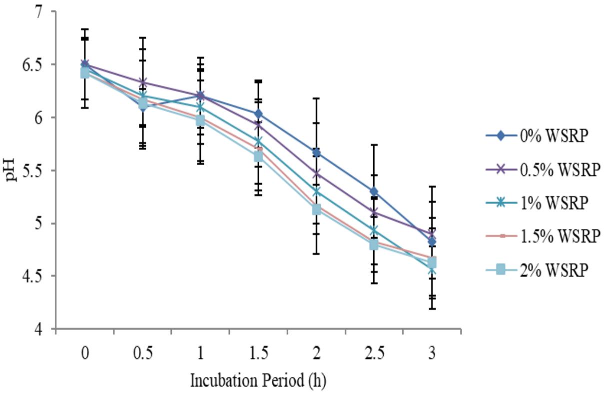

Figure 1a: Changes in pH of WSRP fortified skimmed cow milk during incubation at 42±1°C

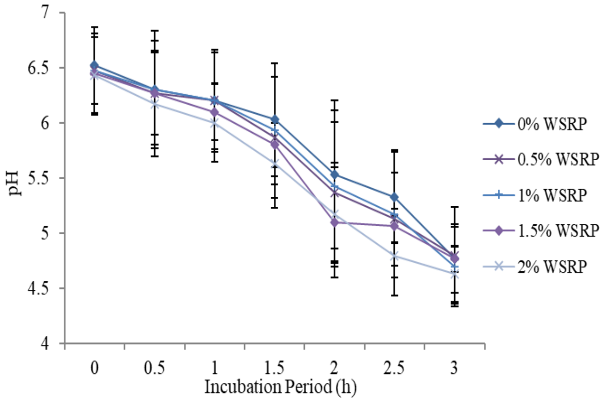

Figure 1b: Changes in pH of WSRP fortified Standardized (3% fat) cow milk during incubation at 42±1°C

reported by Saleem *et al.* (2020) and Mukherjee *et al.* (2020) could have an influence on the slight reduction of pH of the fortified milk compared to the control as reported elsewhere in this article.

During the fermentation, a gradual decrease in pH was observed in all the samples regardless of the type of milk due to the production of lactic acid utilizing lactose in milk by the starter microorganisms. After a 3 h incubation period, pH varied in skimmed milk from 4.57 to 4.9 and in standardized milk from 4.63 to 4.80. A faster drop in pH was observed at 2% WSRP fortification level in both milk types. However, significant differences were not observed ($p>0.05$) in between control and treated samples, even though slight differences were noted. According to the above observations, it can be stated that the addition of WSRP to milk does not hinder the fermentation ability of the yoghurt culture microorganisms, in fact, improved it.

The changes in TA of WSRP fortified pasteurized skimmed and 3% fat standardized cow milk during the incubation period of 3 h at 42±1°C are illustrated in Fig. 2a and Fig. 2b, respectively. During the fermentation, a dramatic increase in TA was observed in control and WSRP fortified pasteurized milk samples inoculated with yoghurt starter culture irrespective of the type of milk. Generally, yoghurt starter culture metabolizes lactose through a homo-fermentative metabolic pathway. Usually, the lactose content is reduced during the fermentation by around 20-30% of the level in the original milk, while the concentration of lactic acid increases (Tamime and Robinson 1999).

Even though a significant difference in pH was not observed during the incubation, a significant ($p<0.05$) difference in the TA was observed between some treatments at specific time points during the incubation. With the increment of the level of WSRP fortification in milk, this difference was also observed to be increased. As mentioned elsewhere in this article, acidic compounds present in WSRP might be one of the reasons for this observation. The addition of plant materials can increase the acidity of milk (do Espirito Santo *et al.* 2012; Veena *et al.* 2015). Also, it can be suggested that the activity of the starter culture might have a favourable impact from the added/ increased level of WSRP. On the other hand, buffering capacity of milk has an impact from the added WSRP extract. Upreti *et al.* (2006) mentioned that the pH buffering capacity of milk comes basically from proteins, free amino acids, weak acids, bases and their complexes with metal cations. Michael *et al.* (2015) experimented with plant extract (a mix of olive, garlic, onion and citrus) supplemented yoghurt and suggested that there were some factors/ingredients in plant extracts which contributed to the greater buffering capacity of supplemented yoghurt.

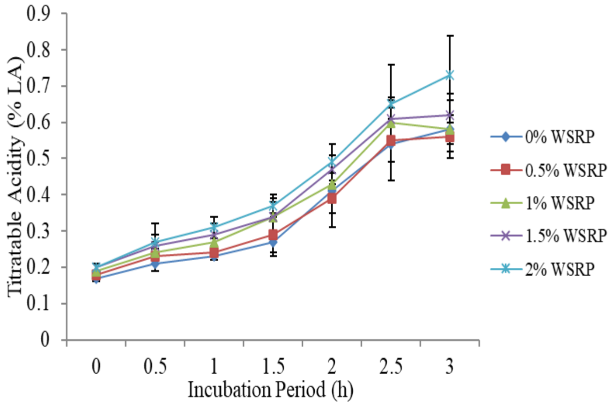

Figure 2a: Changes in titratable acidity of WSRP fortified skimmed cow milk during incubation at 42±1°C

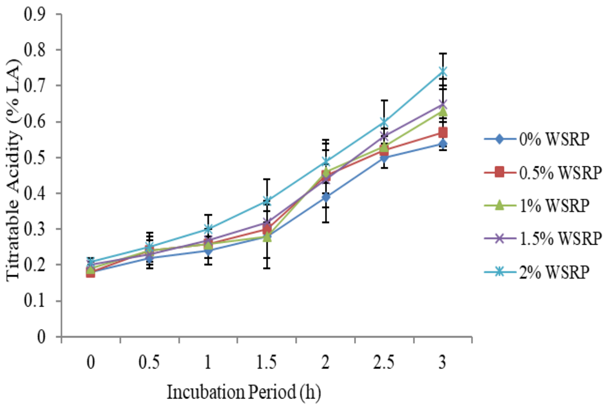

Figure 2b: Changes in titratable acidity of WSRP fortified standardized (3% fat) cow milk during incubation at 42±1°C

CONCLUSION

Fortifying milk with plant materials is of great interest to improve functionality and create functional dairy products with health benefits. This study has shown that the addition of WSRP to the skimmed and 3% fat standardized milk did not cause significant changes in the tested compositional, physicochemical, physical and functional properties except RCT and colour parameters. Rennet coagulation time showed a significantly (p<0.05) decreasing trend with increasing WSRP levels irrespective of the type of milk. Standardized milk had significantly (p<0.05) lower RCT than skimmed milk. Instrumental colour values were significantly (p<0.05) affected by the added WSRP in both milk types. pH was not affected significantly ($p>0.05$) during the incubation even though TA was affected by the addition of WSRP. It shows that there is no negative impact of WSRP fortification on culture microorganisms indicating the possibility of using it in the production of fermented dairy products. Sensory scores were decreased with increasing levels of WSRP in both milk types. Sedimentation of WSRP in fortified milk had a higher impact on reduced sensory scores. From the above results, it is concluded that a possibility exists to fortify milk with WSRP and produce functional fermented dairy products such as cheese and yoghurt. Hence, the information generated would be of great significance in designing new products for the emerging functional dairy products market in Sri Lanka. However, further studies are suggested with WSRP extract to reduce the impact of sedimentation and to observe the active properties of the Ashwagandha herb. This study can be used as a preliminary study for that.

AUTHOR CONTRIBUTION

NMNKN conceptualized and designed the study. AMF performed the experiments. NMNKN and AMF analyzed and interpreted the data. NMNKN drafted and critically revised the manuscript.

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