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Research Application Summary

Development of yoghurt supplemented with selected herbs and analysis of its physico-chemical properties

Mutebi, R.K., Muhumuza, A., Katumba, J. & Mugisha, K.M.

Bishop Stuart University, P.O. Box 9, Mbarara, Uganda
Corresponding author: rmkalibwani@as.bsu.ac.ug

Abstract

With the emergence of fortified foods, there has been a worldwide increase in health awareness and interest in adding herbs as prized food additives in dairy and food products. However the effect of supplementation of dairy products with herbs on their physic-chemical properties is not well documented. This study was done to determine the effect of given amounts of herbal supplementation on the quality and shelf life of yoghurt. The specific objectives of the study were to establish the pH and titratable acidity of the yoghurt supplemented with selected herbs. The selected herbal extracts were Aloe vera (*Aloe barbadensis* Miller), Beetroot (*Beta vulgaris*), and Moringa (*Moringa oleifera*). Herbal yoghurts were developed with different concentrations; 1, 1.5, and 2% each of aloe vera, beetroot and moringa leaf extracts with plain yoghurt used as a control. Yoghurts were stored at 4 °C and their pH and titratable acidity tested on day 1 after incubation, and days 7, and 14 after production and stored at 4°C. The pH of all the samples of aloe vera and beetroot on average was found to decrease between day I to 14, with an increase in titratable acidity, possibly due to increase in microorganism activity and fermentation. This pH range is not only an indicator of good quality yoghurt, but that yoghurt supplemented with the two herbs possibly has more of the beneficial acids for human health as compared to ordinary yoghurt. However, moringa yoghurt at 1% concentration level had nearly the same pH and titratable acidity with plain yorghurt. Higher concentration levels of moringa at 1.5% and 2% were found to have relatively higher pH and less titratable acidity. Although suspected to be richer in minerals than plain yoghurt, its quality could not be guaranteed at that temperature.

Keywords: Moringa, pH, physico-chemical properties, titratable acidity, yoghurt

Resume

Avec l'émergence des aliments enrichis, il y a eu une augmentation mondiale de la sensibilisation à la santé et de l'intérêt pour l'ajout d'herbes comme additifs alimentaires prisés dans les produits laitiers et alimentaires. Cependant, l'effet de la supplémentation des produits laitiers avec des herbes sur leurs propriétés physico-chimiques n'est pas bien documenté. Cette étude a été réalisée pour déterminer l'effet des quantités de suppléments en herbes sur la qualité et la durée de conservation du yaourt. Les objectifs spécifiques de l'étude étaient d'établir le pH et l'acidité du yaourt enrichi avec des herbes sélectionnées. Les extraits de plantes sélectionnés étaient l'Aloe vera (*Aloe barbadensis Miller*), la betterave (*Beta vulgaris*) et le Moringa (*Moringa oleifera*). Des yaourts ont été élaborés avec différentes concentrations : 1, 1,5 et 2 % d'extraits de feuilles d'aloe vera, de betterave et de moringa, le yaourt nature servant de témoin. Les yaourts ont été stockés à 4 °C et leur pH et leur acidité ont été testés le après 1jour l'incubation, et aux jours 7 et 14 après la production et le stockage à 4°C. En moyenne, le pH de tous les échantillons enchi enrichi d'aloe

vera et de betterave a diminué entre le premier et le quatorzième jour, avec une augmentation de l'acidité titrable, probablement due à une augmentation de l'activité des micro-organismes et de la fermentation. Cette plage de pH n'est pas seulement un indicateur de la bonne qualité d'un yaourt, mais aussi du fait qu'un yaourt enrichi avec ces deux plantes contient probablement plus d'acides bénéfiques pour la santé humaine qu'un yaourt ordinaire. Cependant, le yaourt au moringa à un niveau de concentration de 1% avait presque le même pH et la même acidité titrable que le yaourt ordinaire. Des niveaux de concentration plus élevés de moringa, à 1,5% et 2%, on enregistré un pH relativement plus élevé et une acidité titrable moindre. Bien que soupçonné d'être plus riche en minéraux que le yaourt nature, sa qualité ne pouvait être garantie à cette température.

Mots clés : Moringa, pH, propriétés physico-chimiques, acidité titrable, yaourt.

Introduction

Yoghurt is a fermented product made from fresh milk and or reconstituted milk by using bacteria such as *Lactobacillus bulgaricus* and *streptococcus thermophiles*. It may be made with or without any additional food ingredients and permitted food additives (Tamime and Robinson, 1999). Recently there has been an increasing interest in the use of natural food additives and incorporation of health promoting substances into human diet. For this reason, medicinal and culinary herbs are popularly used in food preparation because they contain phytochemicals which impart a variety of health benefits (Exarchau *et al*., 2002). Herbs have been used not just as food flavorings, but also as medicine, additives and preservatives since the ancient times. It is widely acknowledged that there is a strong linkage between food habit and disease prevention; the effects of food on diseases such as diabetes, obesity, osteoporosis, hypertension and cardiovascular disease are documented in literature (WHO, 2002a). At the same time, indigenous knowledge on medicinal plants is gaining worldwide recognition. In this regard, India, the largest producer of medicinal herbs, also referred to as the `Botanical Garden of the World, is acknowledged for its indigenous knowledge and practice in the field of medicinal herbs (Modak *et al*., 2007).

With the emergence of fortified foods, there is a worldwide increase in health awareness and interest in adding herbs as prized food additive in dairy and food products (Ansari, 2012). Due to the increased consumer awareness and interest to follow healthy nutrition and dietary strategy in achieving health benefits from foods beyond their basic nutrition, the market for value-added functional foods has expanded manifold (Kumar *et al*., 2016). The World Health Organization survey (2002b) indicates that about 60% of the world's population and 80% in developing countries depends directly on plants for their medical purposes. These plants are normally rich in phenolic compounds with antioxidant activity which are useful to retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. The phenolic compounds present in spices and herbs might also play a major role in their antimicrobial effects and contribute to their antioxidant and pharmaceutical properties (Cai *et al*., 2004).

In the recent past, traditional dairy products have received special attention from the R&D institutions. However, there is limited literature reported regarding supplementation of herbs into dairy products to improve their functionality. In particular, the physiochemical properties determine the shelf life of a product. Physico-chemical properties of yoghurt have been reported to be affected by the addition of preservatives such as tetra sodium pyrophosphate (TSPP). It reduces the soluble calcium content of the milk and increased casein bound calcium values. It has been found that 0.125%TSPP results in reduction in the turbidity because of micelle dispersion but at 0.15% TSPP there is increase in turbidity due to the aggregation of the casein particles (Ozcan *et al*., 2008). However the effect of supplementation of dairy products with herbs, on their

physic-chemical properties is not well documented; particulary understanding the effect of given amounts of herbal supplementation on the quality and shelf life of yoghurt. The study utilizes Aloe vera (*Aloe barbadensis* Miller), beetroot (*Beta vulgaris*), and moringa (*Moringa oleifera*) as the selected herbal supplements to determine their effect on the shelf life and maintainance of yorghurt. Aloe vera is an ever-green perennial in tropical, semi-tropical and arid climates around the world, cultivated for agricultural and medicinal uses. *Beta vulgaris*, commonly known as beetroot, is the tap root portion of a beet plant also known as beet, table beet, sugar beet, among other names. They are mainly grown for their edible leaves and roots. *Moringa oleifera*, commonly referred to as moringa, is an exceptionally nutritious vegetable with a variety of potential uses ((Devarai *et al*., 2016).

The general objective of this study was to develop and analyze the physico-chemical properties of yoghurt supplemented with selected herbs. The specific objectives were: (i) to develop yoghurt supplemented with different concentration levels of the selected herbs, and (ii) to establish the pH and titratable acidity of the yoghurt supplemented with the selected herbs.

Materials and methods

The study was carried out from the Pharmaceutical Analysis Laboratory of Mbarara University of Science and Technology (MUST). The study used commercial yoghurt starter culture, whole milk, aloe vela gel, moringa leaf, and beet root. Whole milk was purchased from Pearl Dairy farm, Biharwe Mbarara, and the herbs that include aloe vela, beet root, moringa leaf were purchased from Mbarara Zonal Agricultural Research Development Institute (MBAZARDI) of the National Agricultural Research Organisation (NARO). *Lactobacillus acidophilus* and *Streptococcus thermophilus* were used as commercial yoghurt starter culture (Culture Systems, Inc., USA). These samples were transported aseptically to the Pharmaceutical Analysis Laboratory of MUST. The following steps were followed;

Preparation of yoghurt supplemented with the herbs. Herbal yoghurts were developed with different concentrations of beetroot extract (1, 1.5, and 2%), aloe vela gel extract (1, 1.5, and 2%), moringa extract (1, 1.5, and 2%). The yoghurt base made of whole milk was mixed with different herbal extracts (beet root, aloe vela, and moringa), each at the different concentrations (1, 1.5, and 2%). A control sample was made to consist of plain milk, with no herbal supplement. A total of 10 samples was therefore developed. These were then pasteurized at 90°C for 10 min.The pasteurized milk with herbs was cooled and inoculated with 2% v/w commercial yoghurt starter culture containing *L. acidophilus* and *S. thermophilus*. Inoculated milk was incubated at 40 °C for 12 hours and then stored in a refrigerator (4 °C) according to Lee and Lucey (2010) with slight modifications.

Establishing the physico-chemical properties of the herbal yoghurt during incubation. Physico-chemical properties of the herbal yoghurt were established d gyring incubation and storage. Yoghurt samples were incubated at 40°C for 12 hours. The samples were then analysed for pH and titratable acidi y. The pH value and titratable acidity sampled were determined uing a pH meter. Yoghurt samples were then stored for 14 days at 4°C and three days.

Data management and analysis. Data generated were collected and entered into excel software program and analyzed using Stata package version 12 (Statacorp, 2009). A t-test and ANOVA tests were adopted as tests to be used at $P<0.05$.

Results and discussions

The different treatment combinations that were developed during the study, and their labels are shown in Table 1 below.

Table 1. The developed yoghurt supplemented with selected herbs under different treatment combinations

Treatment Label	Treatment Combination
TO	Plain yoghurt mix inoculated with 2% starter culture.
TlA 1	Yoghurt mix inoculated with 2% Starter culture and 1% aqueous aloe vela gel extract
T1A2	Yoghurt mix inoculated with 2% Starter culture and 1.5% aqueous aloe vela gel extract
T1A3	Yoghurt mix inoculated with 2% Starter culture and 2% aqueous aloe vela gel extract
T2B1	Yoghurt mix inoculated with 2% starter culture and 1% aqueous beet root extract
T2B2	Yoghurt mix inoculated with 2% starter culture and 1.5%aqueous beet root extract
T2B3	Yoghurt mix inoculated with 2% starter culture and 2%aqueous beet root extract
T3M1	Yoghurt mix inoculated with 2% starter culture and 1% aqueous moringa leaf extract
T3M2	Yoghurt mix inoculated with 2% starter culture and 1.5%aqueous moringa leaf extract
T3M3	Yoghurt mix inoculated with 2% starter culture and 2%aqueous moringa leaf extract

Physicochemical parameters of yoghurt supplemented with herbs. Values of pH for the developed yoghurt were determined using a pH meter, on different days; 1, 7, and 14 after production. The results are shown in Table 2 below.

The results show that on average, the pH of the samples in the study ranged from 4.11 in aloevera (2%) after day 7 and 14 of analysis, to 4.88 in moringa (2%). For aloe vera and beetroot, these results compare well with the range of 4.0 — 4.6 that was observed for good yoghurt by Masulli (2016). The lower concentration of moringa extract (1%) also falls within this range. The pH of aloe vera and beetroot treatment combinations is on average lower than that of the control, after day one of analysis, and remained so even after 7 and 14 days of analysis. It is likely that the high concentration of essential amino acids present in aloe vera (Sugiastuti *et al*., 2014) and phenolic acids present in beetroot (Antigo *et al*., 2018) contribute to the relatively higher acidity in the two, as compared to plain milk. The reason for acidity and pH decrease might also be due to microorganisms activity and post acidification activity during fermentation. Nontheless, the pH for both remains within the range proposed by Masulli (2016), for good yoghurt. This finding seems to suggest that yoghurt supplemented with aloe vera, beetroot, and a small concentration of moringa, is enriched with more acids that may be beneficial to human health, as compared to ordinary yoghurt, while maintaining a good quality of the yoghurt for at least 14 days. The results also show that the higher the extract concentrations of aloe vera and beet root, the lower the pH suggesting a higher concentration of vital acids in these treatment concentrations, while maintaining good quality.

Table 2. pH values of the developed yoghurt after 1, 7, and 14 days of analysis

Yoghurt treatment combinations	Days of after production		
	1	7	14
TO	4.34	4.32	4.32
T1A1	4.29	4.19	4.15
T1A2	4.24	4.19	4.16
T1A3	4.21	4.11	4.11
T2BI	4.30	4.22	4.21
T2B2	4.22	4.12	4.10
T2B3	4.16	4.14	4.12
T3M 1	4.35	4.30	4.20
T3M2	4.78	4.58	4.59
T3M3	4.88	4.61	4.63

*See Table 1 for details

In the moringa treatment combinations, on the contrary, the results show that the pH of moringa extract concentrations was on average higher than that of the control. Moringa is known to be rich in minerals (Devarai *et al*., 2016) and this might be responsible for the relatively higher pH than ordinary yoghurt. In addition pH increases with increasing moringa extract concentration. These results suggest that at a low moringa leaf extract concentration (1%), yoghurt supplemented with moringa remains good within a period of 14 days, with a possible enhancement of more minerals as compared to ordinary yoghurt. At higher leaf extract concentration however (1.5% and 2%), pH gradually increased beyond 4.6. This result seems to suggest that at high leaf extract concentration, the quality of the supplemented yoghurt is reduced.

Titratable Acidity. The values of tritratable acidity for the developed yoghurt were determined on different days; 1, 7, and 14 after production. The values obtained were an average of the three replicates. The results are shown in Table 3 below.

Table 3. Percentage titratable acidity of the yoghurt treatment compositions

Yoghurt teatments	Days after production		
	1	7	14
Plain yoghurt	0.65	0.68	0.69
Aloe vera yoghurt	0.7810.66	0.8±0.66	0.8±0.66
Beetroot yoghurt	0.7310.66	0.74+0	0.75+0
Moringa yoghurt	0.610.33	0.63+0	0.610.33

Each value is an average of three replicates with Mean ±Standard deviation

Titratable acidity of the control (plain yoghurt) gradually increased with number of days after production. Similarly, titratable acidity of the aloe vera and beetroot yoghurts also increased with number of days after production. Titratable acidity was highest with aloe vera yoghurt ranging from0.78±0.66 after day 1 to 0.8±0.66 after day 14. The results showed that moringa yoghurt had the lowest percentage of titratable acidity of the three selected herbs, and also lower than the control, for all the days of analysis. All treatment effects were significantly different from each other at $P<0.05$. Research conducted by Ashwini (2018) reported that the acidity ranged from 0.64 to 0.79 percent in aloe yoghurt made from milk in India. This range is simillar to that in this study. The reason for titratable acidity increase might be due to the microorganisms' activity, and post acidification during fermentation as discussed for pH above.

Conclusion

The supplementation of yoghurt with aloe vera and beetroot extracts reduced the p1-1 and significantly increased titratable acidity of the resultant yoghurts as compared to ordinary yoghurt. This is a promising indicator for enhanced content of acids that might be vital for human health, improved shelf life and yoghurt maintenance. The supplementation of yoghurt with moringa leaf extract may on the contrary mean more minerals resulting in a more alkaline product. Except at a low concentration (1%), the shelf life and quality of yoghurt supplemented with moringa leaf extract at higher concentration levels may not be guaranteed, requiring more investigation.

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