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Effect of garlic *(Allium sativum)* and ginger *(Zingiber officinale)* powder on chemical composition and sensory property of *Ayib* - Ethiopian cottage cheese

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Abstract

The effect of garlic *(Allium sativum)* and ginger *(Zingiber officinale)* powder on the chemical, microbial and sensory properties of Ayib (an Ethiopian cottage cheese) was investigated. Ayib was made by heating Arera (defatted sour milk) at 50^{0}C for 55 min. Ayib samples of each 1kg were arranged randomly and treated with garlic powder, ginger powder and their mixture (1:1 ratio) at 0, 1, 3 and 5% by weight concentration, respectively and the samples were stored for 10 days at ambient temperature and evaluated every 24 h for total solids, ash, fat, pH, titratable acidity, aerobic mesophilic bacteria, coliform, and yeast and mould counts with three replications. Sensory analysis was also conducted to assess the taste, aroma, color, texture and overall acceptability of all Ayib samples. The General Linear Model procedure of the Statistical Analysis System was used to analyze the data generated. Total solids, ash, fat and titratable acidity of the Ayib samples were not affected ($p>0.05$) by the type as well as level of inclusion of the spices used. A drop in pH value of Ayib treated with 1% garlic and (1 and 5%) ginger powder was observed at the first day of storage, while the pH of Ayib treated with 5% garlic and 5% garlic/ginger powder mixture tended to decline up to the 4th and 3rd day of storage period, respectively. A 3% garlic powder treated samples showed the lowest yeast and mould (6.32 cfu/g) and coliform (1.09 cfu/g) counts. The difference in consumer acceptability of Ayib samples treated with 1 and 5% garlic powder, 1% mixture and the control group (no spice addition) was not marked. Ayib samples treated with 5% garlic powder received the highest consumer acceptability scores compared with other treatments, while garlic powder inclusion at 3% resulted in lower coliform and yeast and mold counts. Understanding the effects of locally available spices, particularly garlic and ginger, on the microbial and biochemical properties as well as on consumer acceptability, is essential in improving traditional fermented milk products.

Introduction

Ayib is a traditional Ethiopian cottage cheese made by heating Arera (defatted sour milk) at 50^{0}C until a distinct curd mass is formed. It is consumed as a side dish with Doro wet (spicy chicken sauce) and Kitfo (minced meat). Ayib comprises about 76-79% moisture content and a pH range of 3.3- 4.6 (Ashenafi, 1992; O' Connor, 1993). The shelf life of Ayib is two days at ambient temperature (30^{0}C) and seven days at 4^{0}C (O'Connor, 1993).

The safety of milk products with respect to food-borne diseases is of great concern around the world. This is especially true in developing countries where processing of milk and milk products takes place under unsanitary and poor production conditions (Ashenafi, 1990). Though, these may contribute to the short shelf life of the products and also result in food poisoning (Ashaye *et al*., 2006); smallholder milk producers in Ethiopia use natural preservatives to improve the quality of milk and milk products (Tolla, 2002; Kassa, 2008; Ayenew *et al*., 2009; Seifu, 2013).

The natural spices, garlic *(Allium sativum)* and ginger *(Zingiber officinale)* are reported to have preservative properties (Belewu *et al*., 2005; Gundogdu *et al*., 2009) and also used as ingredients in making foods (Katzer, 2005; Stoilova *et al*., 2007). As indicated by Mishra and Behal (2010), natural preservatives such as spices can be appropriate alternatives to chemical preservatives used in various food industries minimizing their possible side effects and simultaneously improve the shelf life of food. A number of earlier food science researchers have also reported the positive effects of natural spices, among others, on the sensorial properties of a

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variety of cheeses (Krumov *et al.*, 2010; Licon *et al*., 2012; Hamid and Abdelrahman, 2012). In Ethiopia, however, there is very limited studies conducted so far thus little information is available on the effect of natural spices on microbial, chemical as well as sensory properties of Ayib. This study was, therefore, initiated to evaluate the effect of locally available spices namely garlic *(Allium sativum)* and ginger *(Zingiber officinale)* on microbial, chemical and sensory properties of Ayib.

Materials and Methods

Fresh cow's milk was obtained from Holetta Agricultural Research Center. Matured rhizomes of ginger *(Zinger officinale)* and fresh heads of garlic *(Allium sativum)* were obtained from Jimma and Debre Zeit Agricultural Research Centers, respectively.

Preparation of ginger powder

Fresh mature rhizomes of ginger were separated, thoroughly washed, peeled and sliced (about 2 mm thickness) with sharp knife and sun dried to a final moisture content of 10%. They were then ground into powder using electric kitchen grinder. The powder was sieved with a wire mesh (Sukajang *et al*., 2010).

Preparation of garlic powder

After removing the outer cover, garlic cloves were peeled off; washed with clean water; sliced with sharp knife; and sun dried to a final moisture content of 10%. The slices were then ground into powder using electric kitchen grinder. The powder was sieved with a wire mesh. Both ginger and garlic powders were packed in sterile glass bottles and stored in dry and clean area (Douglas *et al.*, 2005).

Preparation of Ayib

Raw whole milk obtained from 50% crossbred cows (Holstein-Friesian x Boran - Ethiopian local Zebu cattle) was kept at room temperature for 48 h to sour spontaneously. When adequate souring was achieved and the pH of soured milk lowered to 4.0, it was churned by electric churner (SN 0794, Italy) to recover the butter. Then after, the defatted sour milk obtained as a byproduct of butter-making was heated at a temperature of 50^0C for 55 min and cooled overnight. The whey and curd were separated by using a sieve and the curd was retained in the sieve for about an hour for effective drainage.

Treatment arrangement

One kg of Ayib was randomly used for the control treatment (no spice inclusion) and each inclusion level (1, 3 and 5% by weight) of garlic powder, ginger powder, and their mixture (1:1 ratio). Each treatment group was again divide into 10 equal parts (100 g) for chemical, microbial and sensory analysis for 10 storage days. Each treatment was replicated three times.

Storage of Ayib

The Ayib samples treated with ginger powder, garlic powder and their mixture (at 1:1 ratio) - each treatment having three inclusion levels (1, 3 and 5% by weight) were packed in sterile bottles and stored at an average ambient temperature of 22^oC for 10 days.

Total solids content

Total solids were determined by oven drying method. Duplicate 5 g Ayib samples were weighed in pre dried and weighed crucible dishes and dried in an oven (Dv 10621, England) at 110^0C for 24 h. They were then cooled and reweighed. Results were calculated according to AOAC (1995) method 948.12.

Ash content

The dried Ayib samples (from the determination of total solids content) were ignited in a Muffle Furnace at $\leq 550^0$C until the ash was carbon free. Then, it was placed in desiccators for cooling and re-weighed. The initial and final weights of the sample were taken. The weight of the ash observed was divided by the original sample weight and expressed in percent as described by AOAC (1995) method 935.42.

Fat content

The fat content of Ayib samples was determined by Gerber method using the procedure described by Richardson (1985).

pH determination

The pH of the Ayib samples was measured using a digital pH meter after calibrating using standard buffer solutions of pH 4 and 7.

Titratable acidity

A 100 ml of distilled water warmed at 40^0C was added into a flask containing 10 g Ayib sample. It was then stirred vigorously and filtered using filter paper (Whatman No.1). A 25 ml portion of the filtrate that represented 2.5 g of the Ayib sample was titrated with 0.1N NaOH using phenolphthalein as indicator. The result was expressed as % lactic acid and calculated according to AOAC (1995) method 920.124.

Aerobic mesophilic bacteria count (AMBC)

Ayib samples were diluted in 0.1% peptone water (1 g/ml of sample in 9 ml of 0.1% peptone water for initial dilution and by transferring 1 ml of the previous dilution into 9 ml of 0.1% peptone water). AMBC was made by using the surface plate technique of appropriate decimal dilutions of the samples in duplicate on Plate Count Agar (Oxoid, UK) after incubating at 32°C for 24 h. Dilutions were selected so that the total number of colonies on a plate will be between 30 and 250 (Richardson, 1985).

Coliform count (CC)

One gram of Ayib sample was diluted in 9 ml of 0.1% peptone water for initial dilution. Further dilutions were prepared by adding 1 ml of the previous dilution in 9 ml of 0.1% peptone water. Zero point one (0.1) ml of the required dilution was surface plated on duplicate Violet Red Bile Lactose Agar (VRBA) plates and counts were made after incubating the plates at 32°C for 24 h. Dilutions were selected for plate counts of between 15 and 150 colonies. Typical dark red colonies (> 0.5 mm in diameter) were considered as coliforms. This was confirmed by transferring five typical colonies from each plate to tubes of 2% Brilliant Green Lactose Bile Broth (BGLBB). Gas production after 24 hours of incubation at 32°C was considered sufficient evidence for the presence of coliforms (Richardson, 1985).

Yeast and mould count (YMC)

Yeast and mould count was made following recommended standard procedures. Briefly, appropriate dilutions of Ayib samples were surface plated using Potato Dextrose Agar (Oxoid, UK) and incubated for 5 days at 25°C. The number of yeast and mould colonies was then counted.

Sensory analysis

Sensory evaluation of Ayib samples was conducted by consumer panelists according to the method described by Resurrecin (1998). Testing was conducted at the Dairy Technology Laboratory of the Holetta Agricultural Research Center. Fifty-eight adult consumers (23 men and 35 women) were requested to evaluate the sensory attributes of the Ayib samples and fill the questionnaire prepared. Consumer panelists were selected based on the following criteria: age between 18-64 years and they have to be "consumers" of fermented milk products. Ayib samples (20 g) were placed in a three digit coded white plastic plates and served in a bright well ventilated room. Distilled water was provided to the panelists to rinse their mouth after each taste. The sensory attributes of Ayib samples i.e., taste, color, aroma, texture, appearance and overall acceptability were evaluated using a 5-point Hedonic scale (5 = like very much, 4 = like moderately, 3 = neither like nor dislike, 2 = dislike moderately and 1 = dislike very much). Ayib samples were presented randomly.

Statistical analysis

The physicochemical and sensory score data were analyzed using the General Linear Model procedure of the Statistical Analysis System software Version 9.1 and means were separated by Duncan Multiple Range Test.

Results and Discussion

Proximate composition

The chemical composition of Ayib treated with different concentrations of garlic powder, ginger powder and their mixture (1, 3 and 5% g/g) is shown in Table 1. Although there is numerical variations in fat, ash and total solids content of Ayib treated by garlic powder, ginger powder and their mixture compared with the control samples, increasing treatment inclusion levels did not affect the fat, ash and total solids content significantly. This result is in line with reports of earlier studies. Rabita *et al.* (2006), for instance, revealed the non-apparent effect of cardamom, thyme and clove powder addition on moisture, fat, salt and total nitrogen contents throughout the 45 days storage period of white soft cheese made from heated goat's milk. Gundogdu *et al*. (2009), however, reported a significant difference in dry matter content of stirred type yoghurt when garlic was included at various inclusion levels. Values of total solids and ash contents of the control Ayib samples were observed to be the lowest compared with all treated Ayib samples. This might be due to the treatments with different ratio of the spice powders. There was also no significant difference ($p>0.05$) in total solids, ash and fat contents of the Ayib samples throughout the storage period among the four treatments. This could be due to the antimicrobial property of garlic and ginger powder. A similar observation was reported by Oladipo and Jadesimi (2012) where Wara (Nigerian soft cheese) treated with garlic extract showed no reduction in crude protein, fat, ash and moisture contents during storage periods. Alalade and Adeneye (2006), however, reported decreasing fat content of Wara cheese as the storage period advances. Gundogdu *et al.* (2009) also indicated decreasing fat content of garlic treated yoghurt at 1% compared with 0.5% level of inclusion

Table 1. Effect of treatment of ginger and garlic powders and their mixture on proximate composition and acidity of Ayib (Mean ± SE) (n = 10)

Treatment	Fat (%)	Ash (%)	Total solids (%)	pH	Titratable acidity (%lactic acid)
Control	1.35±0.18	1.24±0.09	25.50±1.64	4.29±0.04	0.68±0.08
1%Ginger	1.43±0.28	1.34±0.13	27.2±2.33	4.36±0.03	0.76±0.12
3%Ginger	1.52±0.16	1.34±0.07	27.27±1.29	4.30±0.05	0.79±0.12
5%Ginger	1.32±0.26	1.35±0.04	27.29±1.13	4.33±0.04	0.79±0.17
1%Garlic	1.50±0.22	1.39±0.15	27.02±2.22	4.28±0.05	0.71±0.11
3% Garlic	1.31±0.13	1.35±0.08	27.78±1.36	4.28±0.11	0.77±0.09
5% Garlic	1.18±0.14	1.36±0.06	27.67±1.31	4.30±0.04	0.78±0.16
1%Mixture	1.45±0.25	1.29±0.11	27.62±2.51	4.18±0.09	0.72±0.11
3% Mixture	1.29±0.11	1.34±0.09	27.41±1.91	4.23±0.09	0.73±0.08
5% Mixture	1.16±0.16	1.39±0.05	27.60±1.13	4.37±0.04	0.73±0.14

Means with similar superscript letters in a column are not significantly different (P>0.05)
n = Total number of Ayib samples for each treatment.

Table 2. Microbial counts (Mean ± SE in $\log_{10}$ cfu/g) of Ayib samples treated with different levels of ginger, garlic and their mixture

Treatment	Aerobic mesophilic bacteria count	Yeast and mould count	Coliform count
Control	6.62±0.52	7.43±0.58	1.96±0.37
1%ginger	7.33±0.26	7.35±0.79	1.62±0.36
3%ginger	7.07±0.32	8.25±0.31	2.23±0.78
5%ginger	7.08±0.47	7.82±0.35	2.83±0.53
1%garlic	7.42±0.09	8.01±0.39	2.14±0.15
3% garlic	6.79±0.25	6.32±0.45	1.09±0.47
5% garlic	6.75±0.25	7.32±0.59	1.71±0.16
1%mixture	7.29±0.34	7.82±0.81	2.39±0.58
3% mixture	6.75±0.51	7.85±0.35	2.19±0.59
5% mixture	7.00±0.45	7.20±0.75	2.59±0.21

and as storage time advances.

pH and titratable acidity

Treating Ayib samples with different levels of ginger, garlic and their 1:1 mixture powder did not show significant (p>0.05) effect on the pH value of the samples (Table 1). Similarly, ginger, garlic and their mixture (1:1 ratio) treatment did not show significant effect on titratable acidity of Ayib samples (Table 1). This result is in line with that of Foda *et al.* (2010) who revealed that higher spearmint oil concentration did not affect the titratable acidity of white cheese.

Ayib treated with 1% ginger, 1% garlic and 5% ginger powder showed significant (p<0.001) decrease in pH value at 24 h of storage, while 5% garlic and 5% mixture powder showed a decreasing trend in pH from 0 to 4 and 0 to 3 days of storage period, respectively (Figure 1). The current result agrees with the report of Gundogdu *et al.* (2009) where yoghurt samples treated with 1% garlic showed higher pH value than that with 0.5% during their storage periods. This might be due to the effect of the spices used in retarding the growth of or checking out lactic acid bacteria thereby affecting their activity (Rahman *et al.*, 2006). Then after 3 days, the pH of the Ayib samples tended to gradually increase in all cases except (3 and 5%) ginger powder treated Ayib samples that showed declining trend. Metry *et al.* (2007) showed that the pH of white soft cheese samples treated with cardamom, thyme and clove essential oil significantly (p<0.05) decreased

Table 3. Effect of ginger, garlic and their mixture on consumer acceptability (Mean ± SE) of Ayib samples (n = 58)

Treatment	Taste	Aroma	Color	Texture	Appearance	Overall acceptance
Control	4.95^{a}	4.86^{a}	4.95^{a}	4.91^{a}	4.86^{a}	4.91^{a}
1%ginger	4.03^{cde}	3.94^{cd}	3.97^{cd}	4.19^{bc}	3.87^{de}	4.00^{cd}
3%ginger	4.05^{cde}	4.14^{bcd}	3.73^{de}	3.95^{c}	3.95^{cde}	3.96^{cd}
5%ginger	4.14^{bcd}	4.00^{bcd}	3.95^{cd}	3.95^{c}	3.90^{cde}	3.99^{cd}
1%garlic	4.70^{ab}	4.30^{abcd}	4.70^{ab}	4.33^{cb}	4.30^{bcd}	4.47^{ab}
3% garlic	4.32^{bcd}	4.29^{abcd}	4.36^{cb}	4.14^{bc}	4.25^{bcd}	4.27^{bc}
5% garlic	4.48^{ad}	4.57^{ab}	4.61^{ab}	4.57^{ab}	4.61^{ab}	4.57^{ab}
1%mixture	4.62^{abc}	4.52^{abc}	4.52^{abc}	4.62^{ab}	4.48^{abc}	4.55^{ab}
3% mixture	3.97 ^{de}	4.07^{bcd}	3.43^{de}	4.13^{bc}	4.03^{cd}	3.93^{cd}
5% mixture	3.50 ^{e}	3.77^{d}	3.20^{e}	3.80^{c}	3.43^{e}	3.54^{d}

Means with similar superscript letters in a column are not significantly different (P>0.05)
n = Total number of panelists.

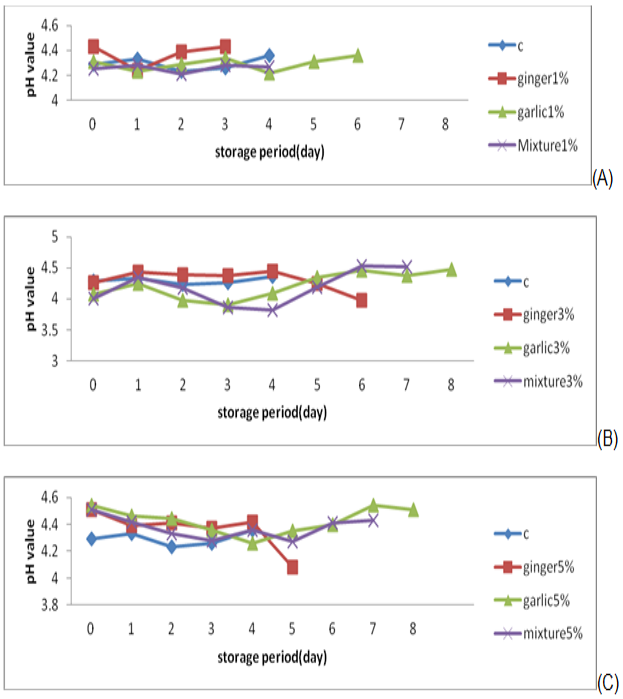

Figure 1. Effect of ginger, garlic and mixture (1:1 ratio of ginger and garlic) powder on pH value of Ayib during storage period of 10 days. (A) 1% inclusion, (B) 3% inclusion and (C) 5% inclusion, C = control

during the pickling period (45 days) in all samples. Moreover, Gundogdu *et al.* (2009) reported that the pH value of garlic treated stirred and set type yoghurts significantly declined during storage period. In the present study, the titratable acidity of the Ayib samples did not show significant change throughout the storage periods, which deviated from the values reported by Osman and Omer (2008) who indicated a significant ($p<0.05$) increase in titratable acidity during 240 days storage time of Sudanese white cheese. A marked ($p<0.05$) increase in titratable acidity of white soft cheese samples treated with cardamom, thyme and clove essential oil during the pickling period (45 days) was also reported (Metry *et al.*, 2007).

Microbial property

Treatment of Ayib with spice powders did not significantly (P>0.05) affect AMBC, YMC and CC of the Ayib samples (Table 2). Though not significant (P>0.05), the lowest counts of yeast and mold and coliform bacteria were observed for Ayib samples treated with 3% garlic powder. As indicated by Paramasivam *et al*. (2007), this might be due the high antimicrobial effect of garlic compared with the other treatments considered.

Consumer acceptability

Consumer acceptability of the Ayib samples was highly affected ($p<0.05$) by the type and level of spice powder inclusion (Table 3). Untreated Ayib samples had the highest taste score among all the samples considered (Table 3). However, Ayib samples treated with 1 and 5% garlic, and 1% mixture powder had comparable taste score with the untreated control sample. One % garlic powder treated Ayib samples had highest taste scores following the untreated samples (Table 3). Untreated Ayib samples had the highest aroma score among all the samples considered (Table 3). However, Ayib samples with 1, 3 and 5% garlic, and 1% mixture powder inclusion had comparable aroma scores with the untreated cheese sample. Five % garlic powder treated Ayib samples got the highest aroma score following the untreated sample (Table 3). Color of untreated Ayib samples had the highest score among the Ayib samples (Table 3). Although, the

untreated Ayib samples had the highest color score, Ayib treated with 1 and 5% garlic, and 1% mixture powder didn't differ significantly in color scores with the untreated Ayib sample (Table 3). Untreated Ayib samples had the highest texture score among all the samples. However, 5% garlic and 1% mixture treated Ayib samples didn't have marked difference in texture score with the untreated Ayib sample. Ayib samples treated with 1% mixture powder had the second highest score for texture following the untreated Ayib sample (Table 3). As it is the case with texture, 5% garlic and 1% mixture (1:1) powder treated Ayib samples didn't significantly differ in appearance score with the untreated cheese sample. Ayib samples treated with 5% garlic powder had the second highest overall appearance score following the untreated Ayib sample (Table 3).

The overall consumer acceptability mean values ranged from 4.91 to 3.54 (Table 3). Untreated Ayib samples had the highest overall consumer acceptability score (Table 3). Ayib samples with 1 and 5% garlic, and 1% mixture powder had comparable acceptance scores with the untreated sample. Five % garlic treated Ayib samples had the second overall acceptability score following the untreated Ayib sample. This result is in line with the report of Gundogdu *et al.* (2009) who revealed that yoghurt samples treated with 1% garlic were more favored than samples treated with 0.5% garlic in both set type and stirred type yoghurts. Ayib samples treated with 5% mixture powder received significantly ($p<0.05$) low mean scores for all the sensory attributes considered.

Conclusion

Ayib samples treated with 5% garlic powder received the highest consumer acceptability scores compared with other treatments. Understanding the effects of locally available spices, particularly garlic and ginger, on the microbial and biochemical properties as well as on consumer acceptability, is essential in improving traditional fermented milk products.

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