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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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Full Length Research Paper

Ethno-culture disparity in food insecurity status: The case of Bullen District, Benishangul-Gumuz Regional State, Ethiopia

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In Ethiopia, a number of studies on food insecurity could be found although they did not explicitly consider ethno-cultural variables while they are very important determinants. The aim of this article was therefore to show the disparity between the Gumuz, Non-gumuz, and the Mixed ethno-cultures in their food insecurity status in Bullen district. Data were collected from 150 sample households and analyzed in an ethno-culture context. The household food balance model was employed to determine their food insecurity status. The results indicated that there were significant differences between ethno-cultures in their food insecurity status. The proportion of food insecure households of Gumuz, Non-gumuz and Mixed ethno-cultures were 62.5, 79 and 48.9%, respectively. Such a disparity existed due to difference in their cultural experiences and traditional values that affect their livelihoods and saving practices, which is also the case throughout Benishangul-gumuz Regional State. Therefore, the study recommends the ethno-cultural approach as appropriate tool for better understanding and addressing the food insecurity challenges in the study area as well as elsewhere.

Key words: Food insecurity, ethno-culture, Bullen, Benishangul-gumuz, Ethiopia.

INTRODUCTION

Ethiopia could not have ensured sustainable economic growth and food security. The recent two-digit economic growth rate the country is merely a superficial report. Poverty and food insecurity are likely to have become the identity of the country. Today, the country is one of the poorest and food insecure nations of the World (Shiferaw et al., 2003; USAID, 2004). Poor performance of agricultural sector, and both policy and non-policy factors are responsible for this (USAID, 2004). Findings from research have confirmed this fact and suggested some policy options (Degefa, 1996; Shiferew et al., 2003; USAID, 2004; Degefa, 2005; Eshetu, 2007; Alem, 2007; Messay, 2009, 2010, 2011, 2013; Guyu, 2011, 2012). For example, the proportion of poor people in the country that was 44% (48% for SSA) in 1990 (USAID, 2004) became 42.2% in 2000 (Brown et al., 2007), as compared to 48% for SSA (USAID, 2004), showing a 2.2% decline over a decade. These became about 60 and 51%, respectively in 2001 (FAO, 2001 cited in Shiferaw et al., 2003). Considering food insecurity alone, the proportion of food insecure population reached 44% in 2003 (USAID, 2004). Moreover, in 2000 the proportion of extremely poor households living below food poverty line of 1650 cal/person/day was 22.5% (Brown et al., 2007) while it was 5.2% in 2012 in Benishangul-gumuz regional state (BGRS) (MoA, 2012).

In BGRS, 58.1% of all households were poor (BGFSS, 2004) and 42% of under-five children were malnourished as compared to 54% in Southern Nations Nationalities and Peoples Region (SNNPR) and 14% in Addis Ababa

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(USAID, 2004). A study of rural food security in Bullen district also revealed 58% food insecure households (Guyu, 2011), a result similar to regional average. Surprisingly, the region is food insecure while it is environmentally stable so that the food insecurity situation can be termed as 'green famine'. The argument of this article is that cultural and hence ethno-cultural variables are important determinants of food security while the role of other factors should not be undermined. Thus, ethno-culture approach (ECA) to food insecurity as both methodological and policy instrument is suggested if a sustainable food security is to be ensured. The aim of the present article, however, is to show the disparity between ethno-cultures of Bullen district in food insecurity status as a validation of the approach for the purpose of universal application. At this juncture one should ask a question about the previous models of foods security: the food availability decline (FAD), food entitlement failure (FED) and sustainable livelihoods approach (SLA)?

Recognizing the importance of ethno-culture approach in assessing food security conditions, it should not be a miracle if studies employing the earlier approach could not solve the challenges of food insecurity. In these studies and approaches, the cultural dimensions have been either entirely overlooked or touched little while their role is substantial. For example, Daskon (2010) clearly shows how cultural traditions can affect people's capability to sustain their livelihoods during times of vulnerabilities to shocks, including food insecurity. It is likely that models and approaches to food security have experienced changes progressively somewhat in a linear fashion since early contributions of classical theorists such as Malthus (1889) and Boserap (1965). The works of Malthus (1889) and later that of Boserap (1965) show the implicit existence of the availability approach until the 1974 World Food Conference (WFC) (Devereux, 2001). Malthus's (1889) work shows how high population adversely affects food supply since land resources are scarce. In contrast, Boserup (1965) rejects the idea of Malthusians that high population places burden on economic growth and maintains that large family size which can increase agricultural productivity through intensive application of labor. Despite their contrasting views, both are likely to implicitly adopt availability approach.

Since 1970s, three major models have been in use for understanding a situation of food security. In the 1970s, FAD was employed to understand food security and food insecurity was understood as a failure in adequate availability of food at global or national levels and researches and policy-makers extensively employed the FAD model (Yaro, 2004). However, it gave way to the FED model in the early 1980s following the works of Sen (1981) emphasizing entitlement (Young et al., 2001; Yaro, 2004). The FED model defines food insecurity as lack of physical and economic access to food. It argues that food availability at macro-level never guarantees households and individuals access to food (Faridi and Waddod, 2010). Later arguing that the FED model is not holistic in nature, the SLA emerged (Scoones, 1998; DFID, 1999; Degefa, 2005; Alinovi et al., 2008). In the SLA, food insecurity is defined as vulnerable livelihoods (Devereux, 2001; Degefa, 2005) because a failure in livelihoods causes food insecurity. Such a shift in models from FAD to EFA and then to SLA is likely a non-stopping process that will proceed (Young et al, 2001). The ECA can be regarded as an aspect of this process of model creation. Although SLA is holistic and captures the dynamic nature of food security, it loosely incorporates culture as human or social capitals. This is the reason for proposing ECA to serve as a viable model for understanding a situation of food security.

MATERIALS AND METHODS

Bullen is one of the 21 districts of Benishangul-gumuz regional state (BGRS) located in Metekel Zone. For clarity, a Zone, in Ethiopia, is an administrative unit lower than a Region but larger than a district whereas, a district is such an administrative unit lower than a zone but larger than a 'kebele' and finally a 'kebele' is the smallest administrative unit lower than a district but larger than a village. Bullen is located north of the Nile (Abay) River which separates it from Yaso district of Kemashi Zone, located south of Abay River. The capital of the district, Bullen town, is located some 103 km from Gilgel Beles, the capital of Metekel Zone to the North, 760 km from Assosa (the regional capital) and 580 km from Addis Ababa to the Northwest. According to Agriculture and Rural Development office of Bullen, the total area of Bullen wereda is about 2,947 km2 (294,127 hectare). Administratively, Bullen district is subdivided into 15 'kebeles' (administrative unit lower than district but larger than village in Ethiopia). While two of these 'kebeles' are parts of Bullen town, the remaining 13 make up the rural 'kebeles'.

Climatically, Bullen district is not uniform throughout. About 95% of the district is dominated by moist 'kola' (a type of humid tropical climate) while the remaining 5% is characterized by 'Weina Dega' (a type of temperate climate). The average annual rainfall of the district ranges from 700 to 1000 mm, whereas the average annual temperature ranges from 23.5 to 35.5°C. Topographically, the general elevation of the district decreases from East and Southeast towards the remaining directions ranging from 1900 to about 900 m a.s.l. in lower areas.

According to Central Statistical Agency [CSA] (2008) of Ethiopia, the total population of the district was estimated at 38,983 (20,026 males 18,958 females). Only 5751 or 14.8% of the population was urban dweller and the remaining 85.2% was rural population. With an estimated area of 2,857.97 sq. km, Bullen had a population density of 13.6 people per sq. km of land. According to the 1994 census result, the major ethnic groups of Bullen woreda are Shinasha (47%), Gumuz (33.5%), Amhara (9.8%), Oromo (8.1%), Agew (0.5%), and others (0.2%). Almost all, the Gumuz people settled in the remote rural areas of the study area. Moreover, the district was dominated by followers of Orthodox Church accounting for 65.1% of the total population. Followers of other faiths are insignificant such as traditional religion (21.5%), Muslims (9.8 %) and others (3.6 %). Figure 1 shows the location of Bullen district at regional and national levels. This ethnic information of the district is the basis for the formulation of the concept of ethno-culture and ECA model in this article.

Accordingly, BGRS consists of a number of ethno-cultures that can be categorized into three, namely the Indigenous, the non-indigenous and the mixed ethno-cultures. In a similar fashion, Guyu (2011) grouped the locally existing ethno-cultures of Bullen district

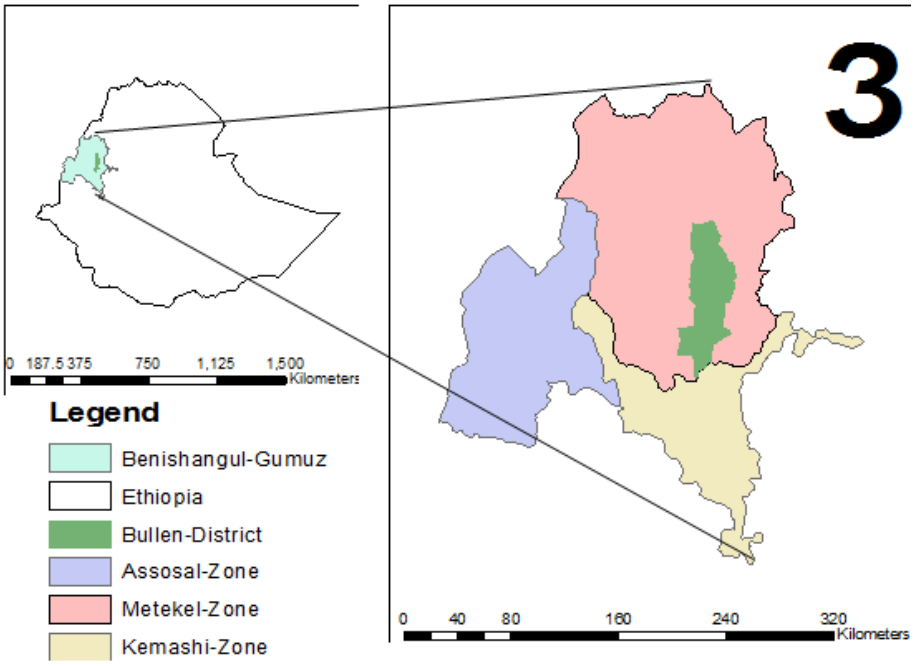

Figure 1. Location of Bullen District at regional and national settings.

into three, namely: the Gumuz (Indigenous), the non-Gumuz (can be indigenous or non-indigenous different from the Gumuz) and the mixed (both Indigenous and Non-indigenous) ethno-cultures. The Gumuz ethno-culture is taken as a distinct culture group because they have still reflecting the traditional ways of living and culture as compared to others, which may have an impact on food insecurity situation. Thus, this article could be considered the first of its kind in explicitly touching the ethno-cultural elements and proposing the ECA and using its idea to analyze the food insecurity situation in Bullen district and to extrapolate it to BGRS as a whole.

Accordingly, a cross-sectional design was employed to generate primary data from a questionnaire-based household survey in order to achieve the objective of the study. As such, a positivist paradigm that pursues rigorous quantitative techniques of data capturing and analyses were suggested to underlie the philosophical assumptions of the study. This was also supplemented by an interpretivist assumption as the limited amount of qualitative data from field observations and photographs were employed. Thus, it can be regarded as a mixed methods approach to food insecurity.

Primary data were collected from 150 randomly selected rural households from all ethno-cultures of the district. The data were, therefore, collected by trained enumerators with close supervision of the researcher. The data gathered were statistically analyzed using software known as Statistical Package for Social Sciences (SPSS) and also manually whenever relevant. Moreover, data from photographs and observation were analyzed qualitatively and used to supplement the quantitative results. The results were presented on tables, interpreted in the context of ethno-culture and extrapolated to BGRS as a whole and conclusions were drawn for the overall ethno-cultures of the region. A household food balance model (HFBM) was also employed to compute food insecurity status of household for all ethno-cultures.

For the purpose of acquiring a reliable data from the respondents, the respondents were guaranteed to be confidential in that any information they would provide should be secrete between them and the author. For ensuring confidentiality, they were informed that the study would be conducted to indicate the culture-related determinants of food insecurity and suggesting a solution for addressing problems of food insecurity better in the study area but for nothing else. Then, the informed consent of the respondents was assured before the actual survey. For this, they were even told to be free so that they can refuse to fill the questionnaire in what they feel uncomfortable. In the researcher's opinion, this had increased the reliability and consistency of the data from the instrument.

RESULTS AND DISCUSSION

As the findings of the study are often presented in tables, the presentation of the results, discussion and interpretation are conducted simultaneously in the context of ethno-cultures of the study area.

Sources of food supply by ethno-culture group

Basically, the economy of rural households in Ethiopia depends on agriculture, be it dominantly pastoral, arable or mixed farming system. Likewise, rural households in Bullen district depend on mixed agriculture where crop production and livestock rearing are simultaneously practiced. Regarding this, Guyu (2011) call the people in BGRS as a semi-pastoral community after observing that livestock production is equally important in the region. The failure of one of these sub-sectors seriously disturbs households' economy and most often results in seasonal food shortage. However, especially in the Gumuz ethno-culture areas where hoe-culture dominates, the cause of food insecurity is more of cultural than failure in farming

Table 1. Major sources of household grain supply by ethno-cultural group.

Sources of grain supply	Distribution of households by sources of grain supply						Total available grain	
	Non-Gumuz		Mixed Area		Gumuz Area			
	Amount (qtl)	Percentage	Amount (qtl)	Percentage	Amount (qtl)	Percentage	Amount (qtl)	Percentage
Own produce	497	85.4	1982.5	95.2	217	78.6	2696.5	91.7
Purchased grain	68	11.7	85	4.1	10	3.6	163	5.5
Borrowed grain	17	2.9	16	0.7	49	17.8	82	2.8
Total	582	100	2083.5	100	276	100	2941.5	100
Households size	38	25.3	96	64	16	10.7	150	100
Qtl/hh	15.3		21.7		17.3		19.61	
Ave hh size	7.34		6.15		6.19		6.45	
Per-capita qtl/year	2.08		3.5		2.7		3.1	

systems. In short, the result of the study showed that the source of food for the surveyed households were own crop produced, purchasing and borrowing from neighbors and relatives (Table 1).

Table 1 shows that there was 2941.5 quintal of grains available for the whole surveyed households during the survey year in all ethno-cultures in the district. The average per capital grain available for the surveyed households in the mixed area (21.7qtl/hh) was better than the Gumuz (17.3 qtl/hh) and the Non-Gumuz (15.3 qtl) areas. However, this does not indicate the food security conditions of each ethno-culture area as it is a gross estimation from which post harvest grain lost and seed reserve should be deduced (which estimates the net dietary energy supply of households).

The overall contribution of own production, purchasing and borrowing accounted for about 91.7, 5.5 and 2.8% of the total annual food grain supply respectively during the survey year. Own production is a leading source of food for all ethno-culture areas. Accordingly, the ethno-culture distribution of each of these sources revealed that the source of grain for 85.4% of the Non-Gumuz, 95.2% of the mixed areas and 78.6% of the Gumuz areas was own produce. Similarly, the source of 11.7% of grain for the Non-Gumuz, 4.1% for Mixed areas and 3.6% for the Gumuz areas was from purchase while that of 2.9% for the Non-Gumuz, 0.7% for mixed areas and 17.8% for the Gumuz areas was from borrowed grain. This implies that food availability dimension is more important source than the access dimension (the entitlement set). However, different ways of interpreting the gross findings in Table 1 can be made.

The larger share of produced grain (95.2%) by the mixed ethno-culture areas obviously tells us that there is a reduced amount of purchased grain (41%) and almost non-existence of borrowed grain (0.7%). However, the lower level of purchasing power of these households may not be taken as an indicator of better agricultural performance as purchasing power itself may emanate from better income from sales of agricultural products. But, from the current figure, the extreme proportion of borrowed grain (0.7%) and the better per capita grain supply (3.5 quintals) may imply that households in mixed areas had practiced agriculture very well than the Non-Gumuz (with per capita quintals of 2.08) and Gumuz households (per capita quintals of 2.7).

Similarly, the larger percentage of purchased grain (11.7%) and smaller amount of borrowed grain (2.9%) in the Non-Gumuz ethno-cultures do not imply better agricultural performance. Relatively higher level of purchasing power of this ethno-culture area is followed by the ethno-culture's ability to adapt with food shortage by practicing non-farm income generating activities (IGAs) as a result, the non-farm IGAs are more diverse in these areas followed by the mixed ethno-cultures and the Gumuz ethno-culture areas.

On the other hand, the larger proportion of borrowed grain (17.8%) in Gumuz ethno-culture areas implies both cultural traditions and the seriousness of the shortage of food supply. Firstly, seasonal food shortage is most frequent and severe among the Gumuz areas than others due to extravagant consumption of grains soon after the post-harvest period, and secondly, there is culture of mutual help through grain borrows or sharing grain stock among same clan of Gumuz community whenever food shortage occurs. Such grain acquisition through grain borrowing and share and extravagant consumption are two popular cultures of the Gumuz ethno-culture that lead to seasonal food shortage in BGR in general and in Bullen district in particular.

Ethno-cultural disparity in dietary energy supply

As mentioned in the methodological section, ordinary HFBM was employed to determine the food insecurity status of households in the study area. As mentioned earlier, not all the food grain produced was consumed or sufficient to support a household throughout the year. Some of the produce could be lost in post-harvest period

Table 2. Net available grain for the sample households for the study year.

Source of grain	Ethno-cultural groups						All cases	
	Non-Gumuz area		Mixed area		Gumuz area			
	Quantity (qtl)	Percentage	Quantity (qtl)	Percentage	Quantity (qtl)	Percentage	Quantity (qtl)	Percentage
Purchased	68	11.7	85	4.1	10	3.6	163	5.5
Borrowed	17	2.9	16	0.8	49	17.8	82	2.8
Own produce	497	85.4	1982.5	95.1	217	78.6	2696.5	91.7
Total	**582**	**100**	**2083.5**	**100**	**276**	**100**	**2941.5**	**100**
PHL (10%)	49.7	8.5	198.3	9.5	21.7	7.9	269.7	9.2
Seed Reserve (5%)	24.9	4.3	99.1	4.8	10.9	3.9	134.8	4.6
Grain sold	17.5	3.1	122	5.9	48.5	17.6	188.0	6.4
NAG	489.9	84.1	1664.1	79.8	194.9	70.6	2349	79.8
No of hhs	38	25.3	96	64.0	16	10.7	150	100
Qtl/hh/year	12.9	-	17.33	-	12.2	-	15.7	-
Ave. hh size	7.34	-	6.15	-	6.19	-	6.5	-
Per capita qtl/year	1.76	-	2.82	-	1.96	-	2.4	-

due to attack by rodents and insects because of improper storage facilities, which might create unexpected depletion of household grain stock. Similarly, some of the grains might be sold for household expenses of certain commodities such as salt, soap, spices, etc while some amount is reserved for seed for the next harvesting season. And some amount might have been borrowed or gained through food aid when shortage occurs. Therefore, the amount of grain sold and losses should be deducted and the amount borrowed or gained through aid must be added to determine the net available grain (NAG) for estimation of daily dietary energy supply (DES) for a particular production year for a household. This can easily be captured using HFBM suggested by Degefa (1996) and later modified and used by several Ethiopian scholars and researchers (Alem, 2007; Degefa, 2005, 2010; Messay, 2009, 2011, 2013; Eshetu, 2007, Guyu, 2011). However, in the study area, food aid was not found to be a source of food grain supply rather only own production, purchasing and borrowing were the principal sources of food grain. In this article, these were considered in the HFBM in order to determine the NAG to a household as follows:

NAG = (OGP + GP + GB) – (GS + SR + PHL)

Where NAG = Net available grain for the household per year; OGP = own grain produce; GS = gain sold; GP = grain purchased; SR = seed reserved; GB = grain borrowed; PHL = post harvest loss.

For simplicity of data collection, many respondents are either reluctant to recall the amount or may roughly guess it, as suggested by Degefa (1996), seed reserve was taken as 5% of total production while that of post-harvest loss was estimated at 10% of total produce. Accordingly, the NAG for the surveyed household for the year was 2349.025 quintals with average of 15.66 quintals /year/hh and per capita quantity of 2.43 quintal/year (Table 2).

The findings on Table 2 show that, the per capita NAG for all households was 2.4 quintal which is well over the average recommended amount (2.25 quintals) for a year. However, there was significant variation between different ethno-cultures. Despite the inter-household variations within respective ethno-culture group, on average there was better NAG (2.83 quintal) in the Mixed ethno-culture areas followed by the Gumuz areas (1.96 quintals) and the Non-Gumuz areas (1.76 quintals). The average grain supply in the mixed areas is well over the nationally recommended per capita annual grain requirement of 2.25 quintal, while the case in Non-Gumuz and Gumuz areas was much lower than the nationally recommended amount. This implies that much has to be done in order to achieve the average grain requirement in Non-Gumuz and Gumuz areas.

Grain produced and food insecurity status of ethno-culture groups

The food insecurity status of households in each ethno-culture area was determined based on the DES calculated from NAG (Table 3). Overall, the proportion of food secure households is much lower (42%) than the food insecure households (52%) in the district. The ethno-cultural distribution shows that the proportion of food secure (21.1%) households is much less than the proportion of food insecure households (78.9%) in the Non-Gumuz ethno-culture area. Similarly, the proportion of food security (51.1.%) is much less than the proportion

Table 3. Duration of feed on grain produced by food security status by ethno-culture group.

Ethno-cultural group		Duration of months of feeding on crop produced								Total	
		Months									
		3-5		6-8		9-11		>=12			
		No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent
Non-Gumuz	FS	0	0	0	0	4	50.0	4	50.0	8	21.0
	FIS	12	40.0	13	43.3	5	16.7	0	0	30	79.0
Mixed Area	FS	1	2.0	2	4.1	21	42.9	25	51.0	49	51.1
	FIS	20	42.6	15	31.8	9	19.2	3	6.4	47	48.9
Gumuz Area	FS	0	0	0	0	1	16.7	5	83.3	6	37.5
	FIS	2	20.0	6	60.0	2	20.0	0	0	10	62.5
All Cases	FS	1	1.6	2	3.2	26	41.3	34	53.9	63	42.0
	FIS	34	39.1	34	39.1	16	18.4	3	3.4	87	58.0
Total		35		36		42		36		150	100
Percent of total		23.3		24.0		28.0		24.0		100	

Table 4. Distribution of sample size in ae, its mean and standard deviation.

Result type	AE in Ethno-cultural area						All cases	
	Non-Gumuz area		Mixed area		Gumuz area			
	Count	Percent	Count	Percent	Count	Percent	Count	Percent
Total	243.43	29.79	491.41	60.15	82.09	10.06	816.93	100
Mean	6.40		5.12		5.13		5.44	
STD	2.63		1.94		2.52		2.25	

of food insecure (48.9%) in the mixed ethno-culture areas while the proportion of food security (37.5%) is much less than the proportion of food insecurity (62.5%) for the Gumuz ethno-culture areas.

Table 3 also shows the duration of feeding on crop produced by the households in each ethno-culture group. One very important finding in this table is that significant proportion of FIS households in all ethno-culture areas feed themselves on own grain production for very short period of time. For example, 40, 42.6 and 20% of FIS households in Non-Gumuz, in the mixed and in the Gumuz ethno-culture areas, respectively, feed their family only for 5 or less months in a year. In a similar fashion, the respective proportion of such FIS households that feed themselves only for 8 or less months was 83.3, 74.4 and 80%. This implies that almost all households in all ethno-culture areas depend on their own produce for much of the year while fulfilling the remaining months (4 months) by either purchasing or borrowing.

Dietary energy supply and food insecurity status of ethno-cultures

Determination of household food insecurity status requires the number of sample population in adult equivalent (AE) because the consumption level of all members of a household is not the same. It varies with age and physical condition of an individual. Table 4 shows the ethno-cultural distribution of sample population in AE.

The number of sample population in AE was 816.93 during the survey year. In other words, 29.79, 60.15 and 10.06% were in the Non-Gumuz, the mixed and the Gumuz areas, respectively. There were variations in the mean size of households in their AE among the three ethno-cultural groups (Table 4). Overall, on average, there were 5.44 people in AE in a household in the district with a standard deviation (TTD) of 2.25. Ethno-culture distribution shows that the mean size of AE in a household was 6.4, 5.12 and 5.13 for the Non-Gumuz, the mixed and the Gumuz ethno-culture, areas respectively. This implies that there was significant difference in the mean size of AE between ethno-cultures with a STD of 2.25 as well as in an intra-household distribution with a higher deviation in Non-Gumuz areas (STD = 2.63) followed by Gumuz areas (STD = 2.52) while the smaller deviation in a mixed areas (STD = 1.94). The implication is that there was a larger deviation in the number of AE from the mean size in the Non-Gumuz followed by the Gumuz and then by the mixed areas. The need for average AE is to calculate the average per capita calorie supply of a household and analyze the difference between ethno-cultures.

Table 5. Daily per capita dietary intake of sample households by sample 'kebeles'.

Sample (kebele)	Size of population and calorie supply		
	Total population (AE)	Total calorie	Mean cal/AE/day
Non-Gumuz	243.43	124329354.60	1399.28
Mixed Area	491.41	516991823.50	2882.35
Gumuz Area	82.09	50162632.00	1674.16
All cases	816.93	691483810.10	2319.02
Mean	5.44	4609892.07	1985.26
STD	2.25	6713384.30	1608.07

Table 6. Household distribution by dietary calorie supply and ethno-culture area.

Calorie supply as per AE/day		Ethno-cultural group						All cases	
		Non-Gumuz area		Mixed area		Gumuz area			
		No.	Percent	No.	Percent	No.	Percent	No.	Percent
<=1050 (50% of NRE)		10	26.3	12	12.5	3	18.8	25	16.7
1050-1575 (50-75% of NRE)		13	34.2	19	19.8	4	25.0	36	24.0
1575-2100 (75-100% of NRE)		7	18.4	16	16.7	3	12.4	26	17.3
2100-2625 (25% more than NRE)		6	15.8	12	12.5	1	6.2	19	12.7
2625-3150 (50% more than NRE)		-	-	13	13.5	2	18.8	15	10
>3150 (more than 50% more NRE)		5.3	7.9	24	25.0	3	18.8	29	19.3
Total		38	100	96	100	16	100	150	100
Total (%)		25.3		64.0		10.7		100	
Food Security Status	< 2100cal (FIS)	30	78.9	47	48.9	10	62.5	87	58.0
	>=2100cal (FS)	8	21.1	49	51.1	6	37.5	63	42.00

The result in Table 5 revealed that the total dietary energy available for all surveyed households was 691483810.10 calorie with daily average per AE of 2319.02 calorie. This indicates that the average daily per capita dietary energy available for the surveyed population constituted 10.4% more than the national average value of 2100 cal/AE/day (Table 5). Without individual, household and ethno-culture differences, this implies that the overall district is food secure. The table shows that the mean per capita calorie supply of all ethno-cultures was 2319.2 calorie. There were significant differences in the mean per capita calorie supply between the ethno-cultures (STD = 1608.07 calorie). The ethno-cultural distribution in the mean per capita calorie supply shows that it was 1399.28, 2882.35 and 1674.16 calorie for the Non-Gumuz, the mixed and the Gumuz ethno-culture areas. This implies that only the mixed culture areas are on average food secure while both the Non-Gumuz and the Gumuz areas are well below the nationally recommended per capita energy required.

The result in Table 6 also revealed that there was disparity among the three ethno-cultural areas in dietary calorie intake and food insecurity status. 26% of households in the Non-Gumuz, 12.5% in the mixed and 16.7% in the Gumuz areas were characterized by calorie supply that is 50% less than the nationally recommended energy (NRE) supply of 2100 kcal. The interest here is to show the proportion of food insecurity status of households in different ethno-culture areas. As such, there were 78.9, 48.9 and 62.5% food insecure households in the Non-Gumuz, mixed and Gumuz ethno-culture areas respectively implying that much attention should be given to Non-Gumuz areas followed by the Gumuz ethno-culture areas than the mixed ones.

Like the case in food insecurity, as can be seen from Table 6 there is also a significant disparity between food secure households of the ethno-cultures (21.1, 51.1 and 37.5% for Non-gumuz, mixed and the Gumuz, respectively).

Household coping strategies by ethno-culture

Wild food as a source of food supply and coping strategy

Hunting and gathering as source of food supply are common practices in the district and in BGRS as a whole especially among the Gumuz community as a coping mechanism. Although the practice is the usual activity, it

Figure 2. A) Young Gumuz going to alcohol market. B) The People at alcohol market.

is most frequent during the periods of food shortage. However, these sources and practices are currently declining due to deforestation of the natural habitat for wild animals, fruits and roots. Households in all ethno-culture areas were asked whether they feed on wildfood by hunting or gathering from wild sources. Only about 30% of them replied that they were feeding on wildfood while the majority of them (70%) did not report that they feed on it. The reason for this, according to them is that currently the sources of wildfood are getting depleted seriously. Besides, hunting animals and destruction of forest is legally forbidden. This seems the reason for hiding the practice of hunting and gathering although according to the researcher's observation almost all Gumuz people of the district and BGR are engaged in gathering wildfood even though hunting is limited activity. But, there are still Gumuz households engaged in hunting of at least wild birds. The statistical analysis revealed that there was positive correlation (r = 0.231) between wild food consumption and food security of the households. But the Chi-square test revealed that its power to differentiate the food secure and food insecure groups was not significant at 0.01 probability level. The main reason for limited hunting, in addition to legal prohibition, is perhaps, due to the fact that the households have already handed over guns by which they hunt wild animals.

Other coping strategies than hunting and gathering

The food-insecure households in all ethno-cultures of the study area were not passive rather used to combat the problems of food shortage through different mechanisms. They are used to engage in numerous activities in order to minimize the impacts of food deficits in the household. Moreover, they used to change the feeding patterns and the amount, frequency and types of meal as well as selling of livestock and household utensils and ornaments such as gold, what Devereaux (2001) in his framework of coping strategies called adaptive strategies and asset disposal strategies respectively. Hunting and gathering wild food and local alcohol retailing as coping activities were reported by the 5.4 and 4.5% respectively from mainly the Gumuz area during the survey period. Whereas, traditional alluvial gold mining (1.8%), was exclusively reported from the Gumuz area. This is because of their access to areas which are rich in alluvial gold. On the other hand, sale of ornaments and engagement in daily labor reported by 6.7 and 2.9% of the respondents were typical coping mechanisms in the Non-Gumuz and the mixed areas. Despite its contribution to households' food security, local alcohol ('Araki') retailing activity was blamed for frequent incidence of food shortage in the Gumuz area and among the Gumuz ethnic groups. The key informants from the district offices and the Non-Gumuz people stated that the Gumuz people wasted much of their time walking long distances for buying 'Araki' even during critical seasons of land cultivation and harvesting crops. This inevitably contributed to the grain deficit for majority of the households among the Gumuz community.

Figure 2 shows young Gumuz with their plastic container for buying 'Araki', a local alcohol for trading (Figure 2A) and ultimately at alcohol market. This photo was taken during critical time (Mid May) of tilling and preparing land for sowing seeds for the next harvesting season. However, the young Gumuz were wandering in the town of Bullen in search of local alcohol ('araki'). Thus, it is easy to guess how much time they are wasting that would be invested in land cultivation.

Conclusion

The result of the study showed the prevalence of food insecurity in the study area where people are perceived to be food secure. Such a perception is due to the fact that there is adequate rainfall and relatively vast virgin land covered by green vegetation. This is because problems associated with food security are diverse, complex and inter-linked so that multidimensional approach is needed to tackle them. The current article was aimed to focus on the ethno-culture differences in the status of food insecurity in Bullen district and then to extrapolate the findings of BGRS as a whole. The article was also aimed at suggesting a new approach (ECA) for future use as an appropriate framework for analyzing food insecurity situations as a general model. The idea of ECA is considered after observing a number of approaches that have been utilized to address food insecurity problems in Ethiopia but none has well addressed the problem. It is, therefore, thought that cultural variables are more important than other environmental and economic factors, or these occur as a function of cultural factors. Promising to formulate and test the ECA model in the forthcoming article by the author, the current article investigated the disparity in the status of food insecurity between different ethno-cultures of Bullen district as a validation of ECA.

Accordingly, there was significant difference in food insecurity status between the three ethno-cultures of the district. There were 79% of food insecure households in the Non-Gumuz ethno-culture areas as compared to 48.9% in the Mixed areas and 62.5% in the Gumuz ethno-culture areas of Bullen district. This disparity can also be inferred to BGRS as the people in the region can be grouped in a similar fashion as those in Bullen district. The indigenous, non-indigenous and the mixed ethno-cultures is appropriate way of grouping and understanding the situation of the region. In conclusion, cultural variables seem to be more important than the environmental and socioeconomic factors for assessing food insecurity situation of BGRS in general and in Bullen district in particular. This is not a blind conclusion but drawn from long-term observation of food insecurity situation (green famine) under green covered environment. The region is well endowed with natural resources and sufficient precipitation. However, people in the region, mainly the indigenous (the Gumuz ethno-culture) suffer most from seasonal food shortage every year. Thus, this article recommends that the future research direction should focus on investigating cultural factors of food insecurity employing the ECA model. This model can capture the ethno-cultural variable directly and the environmental and socioeconomic factors as a function of ethno-culture factors of food insecurity. Thus, as will be shown in the forthcoming article, this model acts as a dynamic one that can holistically capture all dimensions of food insecurity while scaling up the importance of ethno-cultural variables.

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Full Length Research Paper

A two –year seasonal survey of the quality of shea butter produced in Niger state of Nigeria

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Low quality of shea butter has continued to be a major challenge in the shea tree value chain. The quality and identity characteristics of market-ready shea butter produced by family-based processors, the highest contributors to the butter output in Nigeria were studied using standard methods of analysis for two consecutive fruiting years to ascertain the consistency in quality status. This was with the view to determining the suitable market segment the butter could serve. The result generally show significant inconsistencies in both quality and identity parameters within and among the villages and zones studied for the two years. The free fatty acid (ffa), acid value, peroxide value, anisidine value, iodine value, moisture, dirt unsaponifiable matter, saponification value ranged from 5.40±0.14 to 13.45±0.44, 10.50±0.22 to 27.06±0.04, 3.50±0.02 to11.17±0.06, 1.72±0.03 to 4.75±0.08, 38.67±0.68 to 60.37±0.57, 0.56±0.02 to 0.40±0.03, 0.86±0.04 to 1.72±0.01, 5.90±0.04 to 9.27±0.06, 193.0±0.72 to 224.67±0.67 while in the second year, the range were correspondingly 5.26±0.05 to 10.13±0.06, 9.19±0.05 to 20.17±0.12, 2.24±0.01 to 8.07±0.08, 2.08±0.25 to 5.03±0.0.2, 36.97±0.21 to 60.57±0.42, 0.21±0.02 to 0.84±0.03, 0.50±0.03 to 1.46±0.03, 6.60±0.10 to 10.09±0.11 and 189.07±2.06 to 236.5±0.66. The mean values computed for the villages did not approximate the data obtained by pooling and analysing the samples from the respective zones very well. The present status of the butter quality did not describe a particular trend and only suits the local market.

Key words: Fat, shea butter, quality parameters, *Vitelleria paradoxa.*

INTRODUCTION

Fats and oils are important macromolecule component of plant and animal tissue. They provide a more concentrated source of energy than do carbohydrates and proteins (Akoh, 1995). The various fats contribute to the physical and functional properties (solubility, viscosity, rheology, melting behaviour, emulsification, body, creaminess, heat conduction carrier of lipophilic vitamins and flavorants) of most products and nutritional (satiety, calories, essential fatty acids source) and health benefits aspects of food) (Akoh, 1998). Global demand for oils and fats to feed the ever growing world population has continued to grow. For example, since year 2000, when the European Union (EU) allowed chocolate makers to substitute up to 5% of cocoa butter in their chocolate with other vegetable fats such as palm oil or shea butter (Cassiday, 2012), the exports of shea butter have increased dramatically. There are several commercial sources of oils and fats such as shea kernel currently being exploited.

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The Shea tree (*Vitelleria paradoxa)* is multipurpose and highly valued not only for the economic and dietary significance of its cooking oil, but also for the fruit pulp, bark, roots and leaves which are used in traditional medicines and for the wood and charcoal used for building and cooking fuel (USAID, 2005). Shea trees grow wild across a 5000 km wide belt of savannah (Maranz and Wiesman, 2003), (Masters et al., 2004) including most West African countries and further east in Uganda, Sudan and Ethiopia (Chalfi, 2004; Goreja, 2004). Among these countries, Ghana and Burkina Faso are the main shea nut exporters (Walter et al., 2003) while Nigeria with the highest shea tree density is the highest producer although 70% of the shea fruits rot away in the bush due to poor collection mechanism (Adgidzi, 2008). As a natural resource controlled by women and children (Elias and Carney, 2007), the shea tree supports the nutritional, economic and health of the rural families and sustains indigenous plant and animal diversity (The Shea Project, 2008). Processing shea kernel into butter, as a venture, has the potential and capacity to contribute to the world economy and total vegetable fats production when properly harnessed. Shea exports from Africa are now estimated to have grown to an annual maximum of 150,000 t of dry kernel with a current market value of approximately 30 million United State Dollar (USD) with prices around 200 USD per ton free on board West African Port (USAID, 2005). The summary of detailed data on the production and export volume of shea produce of the major west African producers up to 2005 have been provided by Nikiema and Umali (2007).The export market is however strictly regulated by high quality standards for shea butter depending on the user industry (USAID, 2005).

However, due to the fact that shea tree is exploited in the wild, proper and sustainable harnessing is fraught with a lot of challenges beginning from picking of the fruit from the bush through storage to marketing of the products. The problems posed by the methods of collecting shea fruits, processing and the great diversity in the shea fruits result in diverse quality and identity characteristics of the butter produced. These variations in the physico-chemical compositions of vegetable oils have often been attributed to environmental factors such as rainfall, soil fertility, maturation period, agronomic practice and genetic substitution (Maranz et al., 2003; Sonou et al., 2006). The methods of processing shea fruits into nuts and butter specifically varies from family to family and from community to community (some communities add local antioxidants or deodorants during processing to extend the shelf life and reduce the unpleasant odour respectively) (Personal communication), hence the wide variation in the quality of butter from the shea belt (Masters et al., 2004). There are also the problems of the use of inconsistent raw materials (water, shea nut), dirty utensil and work environment (normally under a shea tree), lack of quality control and poor butter storage facilities. Nahm (2011) and Carette et al. (2009) have painstakingly documented the comprehensive list of attendant problems associated with shea kernel processing in Ghana.

At present, shea butter is in high demand in the international market hence attracts a premium price. Generally, according to Food and Agricultural Organization wherever there is a commodity that attracts a premium in the market and has either high value or high-volume sales, there is a propensity for some people to engage in illegal activity in order to make higher profit. These nefarious activities usually involve violating food standards and labelling regulations by misleading the purchaser as to the true nature, substance or quality of the goods demanded. The offence can also take the form of adulteration, which generally involves the dilution of a commodity with less expensive materials or overrate cheaper as if it was a food of greater value (Food and Agricultural Organization). The implication of all this is that food authenticity problems can create enormous harm in the marketplace. These problems include public health problems, defrauding of consumers, dwindling sales of a product when a fraud is detected, distortion in market competition etc (Food and Agricultural Organization).

Although the methods of oils and fats analysis have improved tremendously to a level where overt adulteration and misrepresentation could hardly go undetected, the cheats also devise subtle and sophisticated methods to perpetuate their criminal and detrimental acts. To effectively counter this unwholesome practice, a data bank of typical values of authentic oils and fats is required to enable easy comparison with the samples under investigation in other to confirm the claim or not.

Over the years, there has not been a comprehensive compositional and ancillary data on the Nigerian shea butter and nuts especially what is offered for sale in the market generated within a range of two successive years. Some of the studies carried out earlier were on ad hoc basis and at different times and within small study areas. The history of the sample used in the respective analysis were not comprehensively ascertained and told. Therefore, the sketchy results generated from these studies were difficult to harmonize and less robust to be integrated into other national or regional studies within the West African region. This scenario hinders planning, policy and decision making.

Again, unconfirmed observations of the fruiting cycle show that *Vitellaria paradoxa* give only one good harvest per 3-4 years (Nikiema and Umali, 2007). The local processors understood this cycle event very well hence during the bumper harvest year they pick, process and store the butter to be sold during the lean year (butter for them is more convenient to store than the kernel). In the lean year depending on the availability of fresh shea fruit the processors will either mix the stored butter with freshly processed butter or offer the stored butter as it were for sale in the market. This probably explains why the local processors are usually reluctant to sell freshly

processed butter. The exporter will in turn pool the batches bought from different communities and sources prior to export and so on along the chain. In all this, a new product with poor quality results from these blends. The overriding implication of this practice is the likely dissimilarity in the data on the physico-chemical properties of shea butter sold in the market and those from the point of processing.

This study therefore aimed at surveying the quality and identity parameters of market-ready shea butter processed in Niger State, Nigeria with the view to initiating the development of a databank, eliminating adulteration and ascertaining the highest quality possible in the State. The study will also assist in determining the grades and consistency of shea butter produced by the predominant local processors in Niger State, with the view to helping policy and decision makers as well as the end users.

MATERIALS AND METHODS

Study area

The study area was Niger State, Nigeria. It has the highest shea tree density in Nigeria and a lot of advocacy on quality improvement strategies have been done in the state.

Sample collection

Niger State, Nigeria, is divided into three agricultural or political zones namely A, B and C, probably for administrative convenience. For the purpose of this study, 30 samples of shea butter were purchased from ten villages in each of the agricultural zones. The villages selected at random were Agaie,Koriagi, Dibbo, Badeggi, Kausanagi, Lapai, Egbhanasara, Bida, Gangban and Chiji in zone A; Bassa, Gurmana, Erena, Gwada, Zumba/Shiroro, Gawu, Paiko, Babangida, Kwakuti, Kagara in zone B, and Duku, Auna, Nasko, Pandogari, Rijau, Kaboji, Borgu, Dusai, Warari, Gulbin Boka in zone C. The study was conducted during the peak fruiting season in an attempt to minimize the effect of improper processing conditions or over stored kernels carried over from the previous years.

The set of samples characterized in this work consisted of three samples bought from each of the ten villages of the respective three zones. The samples were bought from the women or sellers that were already on their way to the market and have permanent home addresses. This strategy was necessary because based on personal observations, some of these processors usually take the freshly processed butter home and mixed them with the previously processed or stored batches prior to selling at the market. Therefore, buying the butter from them at the processing shed or point may not portray the true quality state of the butter sold at the market. The samples were bought in 2 L (market-ready) packs of plastic buckets of different colours.

The purchased butter samples were transported to the Biochemistry Division, Nigerian Institute For Oil Palm Research(NIFOR), Benin City, Edo State, Nigeria and stored in an air conditioned room (18-22°C) until the analyses were finished.

In order to simulate approximately what a butter merchant that purchases from these women and equally pooled, equal amount (500 mL) of butter from the ten villages in each zone were respectively pooled and analysed. This sample collection was precisely repeated the following peak fruiting period using the previous processors.

Physicochemical analyses

The purchased butter samples were respectively analysed using the standard method of analysis of AOCS (1997) as follows: free fatty acid (FFA) (Ca-5a), moisture (Ca 2c-25), peroxide value (PV) (Cd 8 -90), saponification value (SV) (Cd-3-25), iodine value(wijs) (IV) (Cd 1-25), specific gravity(Cc10b -25), unsaponifiable matter (UM) (Ca 6a 40) and p-anisidine Value(AnV). Acid value was computed by multiplying the ffa value by 1.99 as oleic acid is the predominant fatty acid in shea butter.

Fatty acid composition

Fatty acid methyl esters were prepared with the shea butter by transesterification using the PORIM Test Method (2004). The fatty acid methyl esters of total lipids were analyzed on a Hewlett Packard HP 6890 gas liquid chromatograph (Palo Alto, CA. USA) equipped with a flame ionization detector and a D-B- wax capillary column (30 m × 0.32 × 0.5 nm) (J & W). The column temperature was programmed from 200°C (held for a minute) to 230°C. The injector temperature was 260°C. The carrier gas nitrogen was set at a flow rate of 3.8 ml per minute. Then, the separated fatty acid methyl esters were identified by comparing their retention times with those of reference samples and quantification was performed with the help of an attached integrator.

Statistical analysis

All chemical analyses were performed in triplicate. The data generated were analysed using the SPSS software version 17. Means and standard deviation were computed. The analysis of variance (ANOVA) $P < 0.05$ was performed to find significant differences between means.

RESULTS AND DISCUSSION

Chemical analysis (quality and identity properties) of shea butter from zones A, B and C are respectively presented in Tables 1a, b and c for year 1 while those of the second year were similarly presented in Tables 2a, b and c respectively. Tables 3, 4, 5 and 6 show respectively the summary of the means of the parameters in the zones for the two years, summary of the mean values of the physico-chemical properties of shea butter for the two years, physico-chemical characteristics of pooled butter for the respective zones and fatty acid composition of the pooled butter for the respective zones.

Dirt

Generally, the dirt contents of the butter samples under consideration were statistically significantly different ($P<0.05$) among all the villages and zones. They were equally outside the specified standards for the international market. The percentage dirt contents in zones for the first year ranged from 0.87±0.01 to 1.71±0.03 for A; 0.86±0.04 to 1.68±0.03 for B while zone C was 0.87±0.03 to 1.72±0.01. In the second year, the ranges of the dirt contents were 0.56±0.02 to 1.12±0.02,

Table 1a. Physico-chemical characteristics of shea butter from Zone A for year (1) 2011.

Parameter	1	2	3	4	5	6	7	8	9	10	Average
FFA	9.47±0.392	8.80±0.290	9.68±0.086	8.57±0.237	5.32±0.251	6.81±0.119	9.74±0.066	6.61±0.087	9.87±0.178	5.4±0.147	8.03±1.76
AV	18.85±0.78	17.51±0.58	19.27±0.17	17.07±0.47	10.59±0.5	13.55±0.24	19.38±0.13	13.16±0.17	19.64±0.35	10.77±0.29	15.98±3.51
DIRT	1.54±0.056	0.87±0.010	0.98±0.025	1.45±0.035	1.45±0.025	1.67±0.074	1.53±0.031	1.71±0.03	1.17±0.02	1.21±0.032	1.36±0.28
MOISTURE	0.06±0.005	0.035±0.001	0.47±0.025	0.5±0.01	0.09±0.001	0.08±0.002	0.43±0.025	0.34±0.45	0.52±0.031	0.08±0.002	0.26±0.23
DENSITY	0.91±0.003	0.91±0.002	0.91±0.002	0.92±0.004	0.90±0.005	0.90±0.002	0.90±0.002	0.91±0.003	0.89±0.01	0.92±0.008	0.9±0.01
PV	4.29±0.015	5.96±0.01	4.45±0.02	5.07±0.02	10.2±0.006	6.75±0.05	8.05±0.038	4.71±0.03	5.8±0.34	4.68±0.04	6.0±1082
AnV	3.76±0.015	4.12±0.015	3.7±0.026	2.94±0.017	4.64±0.071	3.86±0.02	2.98±0.025	2.01±0.025	2.72±0.078	2.08±0.025	3.28±0.84
SV	236.5±0.66	206.83±4.65	215.67±4.04	202.67±2.52	189.83±3.84	193.07±0.95	222.7±2.21	208.67±2.29	196.57±1.56	216.33±0.91	208.88±14.06
IV	40.83±0.21	38.67±0.68	50.53±0.42	42.93±0.31	39.37±0.42	52.63±0.25	40.97±0.50	39.6±0.66	55.77±0.35	45.37±0.40	44.67±5.97
UM	6.84±0.067	7.35±0.093	7.61±0.16	6.86±0.074	7.83±0.031	7.62±0.199	5.98±0.025	6.14±0.057	8.85±0.015	9.10±0.99	7.42±0.99
MP	31 – 36	32 – 38	33 – 38	30 – 37	30 – 35	31 – 37	33 – 35	34 – 38	31 – 37	31 – 36	
ANOVA	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

Villages*:* 1=Agaie; 2=Koriagi; 3=Dibbo; 4=Badeggi; 5=Kausanagi; 6=Lapai; 7=Egbhanasara; 8=Bida; 9=Gangban;10 Chiji. Abbreviations/Units: FFA=Free fatty acid, %; Density at 40°C g per mL; Moisture, %; Dirt, %; SV = Saponification value, mg KOH/g; IV=Iodine value, g iodine/100-g sample (WIJS method); UM=unsaponifiable matter, g/kg; AV=Acid value, mg KOH/g; PV=peroxide value, meq oxygen/100-g sample, AnV= Anisidine value (Anv) mg/kg, MP=melting point,°C.

Table 1b: Physico-chemical characteristics of shea butter from Zone B for year (1)2011.

Parameter	1	2	3	4	5	6	7	8	9	10	Average
FFA	13.6±0.021	13.6±0.02	13.45±0.44	6.71±0.22	8.07±0.03	8.76±0.4	12.24±0.24	10.18±0.36	8.44±0.05	9.0±0.08	10.39±2.49
AV	26.67±0.04	27.06±0.04	26.77±0.88	13.35±0.44	16.07±0.05	17.44±0.79	24.36±0.47	20.26±0.71	16.8±.010	17.9±0.15	0.55±0.04
DIRT	1.28±0.03	1.05±0.03	1.22±0.07	1.59±0.03	1.38±0.04	0.97±0.07	1.68±0.03	1.40±0.06	0.86±0.04	1.42±0.02	1.28±0.26
MOISTURE	0.08±0.007	0.42±0.03	0.33±0.068	0.50±0.034	0.41±0.021	0.47±0.01	0.29±0.022	0.36±0.044	0.36±0.024	.41±0.048	0.36±0.12
DENSITY	0.92±0.004	0.91±0.003	0.82±0.231	0.91±0.002	0.99±0.006	0.95±0.006	0.95±0.003	0.95±0.003	0.90±0.004	0.95±0.014	0.92±0.08
PV	5.66±0.004	6.55±1.37	4.67±0.04	6.82±0.03	8.34±0.006	9.12±0.03	10.17±0.17	7.61±0.16	8.19±0.03	11.17±.06	7.83±1.93
AnV	3.42±0.006	2.7±0.017	3.10±0.015	2.75±0.025	2.81±0.046	1.90±0.006	3.53±0.035	2.96±0.057	3.62±0.327	4.19±0.183	3.1±0.62
SV	201.13±0.64	190.17±0.25	211.43±0.85	195.13±1.5	230.63±0.56	215.5±0.6	195.6±0.4	214.9±1.05	198.77±0.47	202.5±3.15	205.58±11.95
IV	51.3±0.36	59.9±0.15	49.5±0.53	60.37±0.57	41.2±0.3	45.8±0.363	56.07±0.49	59.87±0.12	44.2±.3	48.5±0.53	51.67±6.79
UM	7.94±0.06	8.33±0.06	9.12±0.13	6.82±0.03	7.63±0.17	8.33±0.08	9.43±0.06	5.93±0.04	7.34±0.07	6.11±0.63	7.70±1.15
MP	31 – 34	32 – 36	32 – 38	31 – 35	33 – 36	34 – 38	34 – 36	33 – 35	30 – 35	31 – 35	
ANOVA	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

Villages:1=Bassa; 2=Gurmana; 3=Erena; 4=Gwada; 5=Zumba/Shiroro; 6=Gawu; 7=Paiko; 8=Babangida; 9=Kwakuti;10=Kagara **.** Abbreviations/Units: FFA=Free fatty acid, %; Density at 40°C g per mL; Moisture, %; Dirt, %; SV = Saponification value, mg KOH/g; IV=Iodine value, g iodine/100-g sample (WIJS method); UM=unsaponifiable matter, g/kg; AV=Acid value, mg KOH/g; PV=peroxide value, meq oxygen/100-g sample, AnV= Anisidine value (Anv) mg/kg, MP=melting point,°C.

Table 1c. Physico-chemical characteristics of shea butter from Zone C for year (1)2011.

Parameter	1	2	3	4	5	6	7	8	9	10	Average
FFA	11.82±0.10	10.45±0.05	12.12±0.03	8.9±0.01	9.65±0.07	11.24±0.03	5.28±0.12	6.8±0.02	10.7±0.05	9.34±0.02	9.63±2.10
AV	23.53±0.19	20.79±0.1	24.11±0.06	17.7±0.02	19.21±0.13	22.36±0.05	10.5±0.22	13.54±0.03	21.3±0.1	18.59±0.04	0.64±0.02
DIRT	1.62±0.03	1.34±0.01	1.29±0.02	1.56±0.02	1.02±0.02	0.99±0.01	0.87±0.03	1.72±0.01	1.54±0.02	1.65±0.02	1.36±0.3
MOISTURE	0.09±0.01	0.09±0.01	0.38±0.02	0.48±0.03	0.41±0.02	0.56±0.03	0.4±0.03	0.38±0.01	0.6±0.02	0.37±0.02	0.38±0.17
DENSITY	0.89±0.003	0.10±0.003	0.94±0.004	0.94±0.002	0.97±0.003	0.96±0.003	0.98±0.002	0.99±0.001	0.9±0.004	0.9±0.005	0.95±0.04
PV	8.44±0.01	5.95±0.02	10.22±0.03	9.45±0.10	7.26±0.06	7.79±0.04	8.9±0.02	5.5±0.04	3.5±0.02	6.35±0.07	7.34±1.97
AnV	3.56±0.03	2.96±0.02	3.49±0.01	2.87±0.02	1.75±0.02	3.49±0.01	4.55±0.10	3.96±0.02	5.03±0.02	3.36±0.01	3.5±0.88
SV	200.1±0.8	197.77±1.62	210.2±0.92	232.4±1.71	209.93±0.47	189.07±2.06	198.3±0.62	221.27±1.59	204.97±0.50	2.3.2±0.56	206.72±12.15
IV	45.23±0.35	39.8±0.1	43.3±0.92	45.47±1.58	50.73±0.40	43.87±0.38	58.63±0.38	48.07±0.76	40.97±0.21	39.63±0.67	45.57±5.62
UM	8.38±0.04	6.98±0.05	7.95±0.09	6.87±0.06	8.08±0.07	7.93±0.06	8.98±0.04	7.77±0.07	8.89±0.02	9.27±0.06	8.12±0.77
MP	32 - 37	33 – 37	29 – 35	32 – 35	33 – 36	30 – 35	33 – 35	30 – 34	32 – 36	33 – 35	
ANOVA	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

Villages: 1=Duku; 2=Auna; 3=Nasko; 4=Pandogari; 5=Rijau; 6=Kaboji; 7=Borgu; 8=Dusai; 9=Warari; 10=Gulbin Boka. Abbreviations/Units: FFA=Free fatty acid, %; Density at 40°C g per mL; Moisture, %; Dirt, %; SV = Saponification value, mg KOH/g; IV=Iodine value, g iodine/100-g sample (WIJS method); UM=unsaponifiable matter, g/kg; AV=Acid value, mg KOH/g; PV=peroxide value, meq oxygen/100-g sample, AnV= Anisidine value (Anv) mg/kg, MP=melting point,°C.

Table 2a. Physico-chemical characteristics of shea butter from Zone A for year (2)2012.

Parameter	1	2	3	4	5	6	7	8	9	10	Average
FFA	9.04±0.09	9.50±0.06	9.67±0.03	8.5±0.05	7.41±0.05	6.72±0.03	9.6±0.02	9.4±0.03	8.6±0.03	7.32±0.09	8.58±1.04
AV	18.0±0.18	18.91±0.13	19.25±0.06	16.92±0.10	14.75±0.10	13.37±0.10	19.1±0.04	18.71±0.06	17.12±0.06	14.57±0.17	1.06±0.03
DIRT	0.56±0.02	1.46±0.03	1.2±0.02	0.97±0.03	0.90±0.06	0.57±0.04	1.12±0.02	0.85±0.02	0.93±0.03	0.88±0.01	0.94±0.26
MOISTURE	0.43±0.02	0.53±0.01	0.49±0.02	0.49±0.04	0.46±0.03	0.29±0.02	0.4±0.01	0.44±0.01	0.36±0.02	0.41±0.03	0.43±0.07
DENSITY	0.92±0.003	0.91V0.006	0.91±0.004	0.93±0.003	0.92±0.002	0.96±0.054	0.92±0.003	0.92±0.003	0.91±0.003	0.92±0.002	0.92±0.02
PV	4.67±0.05	5.68±0.04	6.36±0.07	4.38±0.05	3.91±0.06	3.7±0.05	3.9±0.02	4.09±0.03	5.29±0.01	4.9±0.04	4.69±0.84
AnV	2.19±0.02	2.36±0.02	2.17±0.03	1.86±0.03	3.23±0.02	3.32±0.02	1.97±0.03	2.51±0.03	1.93±0.02	2.09±0.02	2.36±0.50
SV	224.67±0.67	199.37±0.64	201.93±0.65	196.3±0.76	199.53±0.90	209.2±0.9	197.3±0.36	213.4±0.61	209.6±0.35	198.87±0.15	205.02±8.76
IV	39.43±0.68	40.67±0.15	50.7±0.46	49.6±0.61	48.73±0.76	46.97±.21	43.77±0.47	50.83±1.02	48.07±0.61	42.37±0.65	46.11±4.11
UM	9.27±0.03	7.1±0.01	8.19±0.03	7.65±0.15	8.29±0.04	9.14±0.05	8.94±0.06	6.93±0.07	7.2±0.04	6.72±0.92	7.94±0.92
MP	33 – 36	34 – 38	31 – 35	32 – 35	31 – 35	33 – 36	31 – 34	34 – 38	31 – 35	32 – 36	
ANOVA	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

Villages: 1=Agaie; 2=Koriagi; 3=Dibbo; 4=Badeggi; 5=Kausanagi; 6=Lapai; 7=Egbhanasara; 8=Bida; 9=Gangban;10 Chiji. Abbreviations/Units: FFA=Free fatty acid, %; Density at 40°C g per mL; Moisture, %; Dirt, %; SV = Saponification value, mg KOH/g; IV=Iodine value, g iodine/100-g sample (WIJS method); UM=unsaponifiable matter, g/kg; AV=Acid value, mg KOH/g; PV=peroxide value, meq oxygen/100-g sample, AnV= Anisidine value (Anv) mg/kg, MP=melting point,°C.

Table 2b. Physico-chemical characteristics of shea butter from Zone B for year (2)2012.

Parameter	1	2	3	4	5	6	7	8	9	10	Average
FFA	7.50±0.20	8.0±0.04	6.85±0.06	8.36±0.04	4.95±0.06	5.76±0.24	8.68±0.02	10.13±0.06	9.44±0.06	8.18±0.04	7.78±1.53
AV	14.69±0.03	15.85±0.08	13.64±0.13	16.64±0.08	9.85±0.12	11.46±0.49	17.28±0.05	20.17±0.12	18.78±0.11	10.27±0.08	1.12±0.02
DIRT	1.34±0.02	1.03±0.01	1.06±0.03	1.13±0.02	1.09±0.01	1.06±0.01	0.99±0.01	0.5±0.03	1.15±0.02	1.19±0.02	1.05±0.21
MOISTURE	0.54±0.03	0.45±0.02	0.48±0.01	0.63±0.02	0.36±0.02	0.64±0.01	0.56±0.02	0.49±0.01	0.84±0.03	0.69±0.02	0.57±0.13
DENSITY	0.900±0.002	0.900±0.003	0.940±0.007	0.910±0.006	0.990±0.007	0.910±0.002	0.920±0.001	0.970±0.002	0.920±0.002	0.940±0.005	0.920±0.03
PV	7.31±0.02	6.44±0.03	5.65±0.02	4.51±0.09	4.3±0.02	3.99±0.02	7.25±0.05	6.58±0.03	3.79±0.04	4.09±0.02	5.39±1.39
AnV	2.62±0.30	3.86±0.04	4.63±0.12	3.27±0.06	3.06±0.03	4.15±0.05	3.55±0.06	4.75±0.08	3.99±0.02	3.48±0.05	3.74±0.66
SV	193.0±0.72	212.8±0.66	199.0±0.7	202.43±1.62	207.67±0.40	198.03±0.64	210.53±0.25	200.0±0.49	219.47±0.9	216.9±0.56	204.99±8.52
IV	50.6±0.53	36.97±0.21	40.97±0.31	40.33±0.84	52.7±0.3	51.1±0.62	46.2±0.56	45.07±0.67	57.57±0.25	40.07±1.10	45.56±5.56
UM	9.14±0.02	8.93±0.09	7.01±0.08	7.61±0.33	9.58±0.11	6.91±0.17	8.96±0.08	8.6±0.05	7.47±0.04	9.11±0.03	8.33±0.95
MP	31 – 34	33 – 35	32 – 36	33 – 38	30 – 35	31 – 35	29 – 34	31 – 34	33 – 35	33 – 35	
ANOVA	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

Villages:1=Bassa; 2=Gurmana; 3=Erena; 4=Gwada; 5=Zumba/Shiroro; 6=Gawu; 7=Paiko; 8=Babangida; 9=Kwakuti;10=Kagara **.** Abbreviations/Units: FFA=Free fatty acid, %; Density at 40°C g per mL; Moisture, %; Dirt, %; SV = Saponification value, mg KOH/g; IV=Iodine value, g iodine/100-g sample (WIJS method); UM=unsaponifiable matter, g/kg; AV=Acid value, mg KOH/g; PV=peroxide value, meq oxygen/100-g sample, AnV= Anisidine value (Anv) mg/kg, MP=melting point,°C.

Table 2c. Physico-chemical characteristics of shea butter from Zone C for year (2)2012.

Zone C (2)	1	2	3	4	5	6	7	8	9	10	Average
FFA	8.48±0.11	7.72±0.06	8.3±0.03	7.51±0.07	5.42±0.04	5.92±0.02	8.37±0.03	4.62±0.03	8.74±0.02	5.26±0.05	7.03±1.51
AV	16.88±0.22	15.36±0.12	16.52±0.09	14.94±0.14	10.79±0.07	11.78±0.04	16.65±0.06	9.19±0.05	17.40±0.03	10.47±0.01	1.07±0.02
DIRT	1.26±0.03	0.55±0.04	0.64±0.02	0.73±0.02	1.06±0.03	1.12±0.02	1.07±0.02	0.97±0.03	0.87±0.03	1.26±0.01	0.95±0.24
MOISTURE	0.69±0.02	0.36±0.03	0.67±0.03	0.54±0.02	0.48±0.02	0.23±0.02	0.21±0.02	0.72±0.002	0.41±0.002	0.54±0.02	0.48±0.18
DENSITY	0.9±0.003	0.96±0.049	0.92±0.05	0.92±0.002	0.90±0.003	0.91±0.002	0.91±0.002	0.9±0.002	0.93±0.055	0.91±0.003	1.19±1.5
PV	2.24±0.01	3.26±0.05	3.67±0.02	2.99±0.03	5.67±0.03	4.97±0.03	3.74±0.07	5.22±0.03	4.37±0.06	8.07±0.08	4.47±1.54
AnV	3.17±0.02	2.25±0.03	1.99±0.03	1.72±0.03	2.91±0.03	3.16±0.05	2.81±0.05	2.96±0.03	1.86±0.04	2.63±0.03	2.55±0.53
SV	205.43±1.04	199.4±0.7	216.3±0.96	221.67±0.75	203.17±0.93	213.23±0.76	196.83±0.15	201.47±1.44	206.4±0.96	202.77±3.76	206.67±7.75
IV	49.7±0.56	`51.53±2.05	39.03±0.45	41.7±0.44	43.9±0.1	51.6±0.35	55.6±0.27	60.57±0.42	56.7±0.36	55.97±0.71	50.63±6.86
UM	6.96±0.03	7.98±0.05	8.0±0.10	9.49±0.11	7.91±0.07	10.09±0.11	8.58±0.16	7.96±0.06	6.6±0.10	8.54±0.08	8.21±1.01
MP	33 – 37	31 – 35	33 – 38	33 – 35	31 – 34	32 – 34	33 – 35	33 – 36	33 – 35	34 – 36	
ANOVA	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

Villages: 1=Duku; 2=Auna; 3=Nasko; 4=Pandogari; 5=Rijau; 6=Kaboji; 7=Borgu; 8=Dusai; 9=Warari; 10=Gulbin Boka. Abbreviations/Units: FFA=Free fatty acid, %; Density at 40°C g per mL; Moisture, %; Dirt, %; SV = Saponification value, mg KOH/g; IV=Iodine value, g iodine/100-g sample (WIJS method); UM=unsaponifiable matter, g/kg; AV=Acid value, mg KOH/g; PV=peroxide value, meq oxygen/100-g sample, AnV= Anisidine value (Anv) mg/kg, MP=melting point,°C.

Table 3. Summary of the means of the parameters in the zones for the two years.

Parameter	2011				2012			
	Zone A	Zone B	Zone C	Remark	Zone A	Zone B	Zone C	Remark
FFA	8.03±1.76	10.39±2.49	9.63±2.10	SIG	8.58±1.04	7.78±1.53	7.03±1.51	SIG
AV	15.98±3.51	0.55±0.04	0.64±0.02	SIG	1.06±0.03	1.12±0.02	1.07±0.02	SIG
DIRT	1.36±0.28	1.28±0.26	1.36±0.3	NS	0.94±0.26	1.05±0.21	0.95±0.24	NS
MOISTURE	0.26±0.23	0.36±0.12	0.38±0.17	SIG	0.43±0.07	0.57±0.13	0.48±0.18	SIG
DENSITY	0.9±0.01	0.92±0.08	0.95±0.04	SIG	0.92±0.02	0.92±0.03	1.19±1.5	NS
PV	6.0±1082	7.83±1.93	7.34±1.97	SIG	4.69±0.84	5.39±1.39	4.47±1.54	SIG
AnV	3.28±0.84	3.1±0.62	3.5±0.88	NS	2.36±0.50	3.74±0.66	2.55±0.53	SIG
SV	208.88±14.06	205.58±11.95	206.72±12.15	NS	205.02±8.76	204.99±8.52	206.67±7.75	NS
IV	44.67±5.97	51.67±6.79	45.57±5.62	SIG	46.11±4.11	45.56±5.56	50.63±6.86	SIG
UM	7.42±0.99	7.70±1.15	8.12±0.77	SIG	7.94±0.92	8.33±0.95	8.21±1.01	NS

Abbreviations/Units: FFA=Free fatty acid,%; Density at 40 ºC g per mL; Moisture, %, Dirt, %;SV = Saponification value, mg KOH/g; IV=Iodine value, g iodine/100-g sample (WIJS method),UM=Unsaponifiable matter, g/kg; AV=Acid value, mg KOH/g; PV=Peroxide value, meq oxygen/100-g sample, AnV= Anisidine value (Anv) mg/kg. SIG= significantly difference (P<0.05); NS=not significant.

Table 4. Summary of the mean values of the physico-chemical properties of shea butter for the two years.

Parameter	2011	2012	REMARK
FFA	9.35±2.34	7.80±1.50	SIG (P>0.05) There is difference
AV	18.6±4.65	15.51±3.0	SIG (P<0.05) There is difference
DIRT	1.34±0.278	0.98±0.24	SIG (P<0.05)There is difference
MOISTURE	0.33±0.18	0.49±0.15	SIG (P<0.05) There is difference
DENSITY	0.93±0.05	1.01±0.87	NS (P>0.05) There is no difference
PV	7.05±2.04	4.85±1.33	SIG(P<0.05) There is difference
AnV	3.29±0.8	2.88±0.83	SIG (P<0.05) There is difference
SV	207.06±12.69	205.56±8.3	NS (P>0.05) There is no difference
IV	47.30±6.83	47.43±6.01	NS (P>0.05) There is no difference
UM	7.74±1.01	8.16±0.97	SIG (P<0.05) There is difference

Abbreviations/Units: FFA=Free fatty acid, %; Density at 40°C g per mL; Moisture, %; Dirt, %; SV = Saponification value, mg KOH/g; IV=Iodine value, g iodine/100-g sample (WIJS method); UM=unsaponifiable matter, g/kg; AV=Acid value, mg KOH/g; PV=peroxide value, meq oxygen/100-g sample, AnV= Anisidine value (Anv) mg/kg.

Table 5. Physico-chemical characteristics of pooled butter for the respective zones.

Parameter	2011			2012		
	Zone A	Zone B	Zone C	Zone A	Zone B	Zone C
FFA	9.63±0.03	12.41±0.02	11.24±0.01	8.23±0.02	8.02±0.01	7.84±0.02
AV	19.16±0.04	24.70±0.04	22.37±0.03	16.38±0.04	15.96±0.02	15.60±0.02
DIRT	1.10±0.04	1.42±0.02	1.24±0.02	0.91±0.03	0.98±0.02	0.96±0.03
MOISTURE	0.31±0.03	0.39±0.03	0.41±0.02	0.44±0.02	0.59±0.03	0.52±0.03
DENSITY	0.911±0.03	0.923±0.02	0.909±0.02	0.896±0.01	0.914±0.03	0.897±0.03
PV	7.29±0.05	8.85±0.04	7.76±0.03	5.27±0.07	6.14±0.04	5.34±0.04
AnV	2.78±0.04	2.49±0.05	2.47±0.03	1.95±0.05	3.44±0.05	2.84±0.03
SV	196.4±0.03	198.2±0.03	195.4±0.03	198.74±0.02	200.1±0.02	196.5±0.03
IV	41.27±0.03	36.85±0.02	39.43±0.03	36.67±0.03	38.46±0.05	40.29±0.04
UM	6.43±0.03	7.10±0.03	6.57±0.04	5.84±0.02	6. 92±0.02	6.48±0.03
ANOVA	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

Table 6. Fatty acid composition of the pooled butter for the respective zones.

Fatty acid	2011			2012		
	Zone A	Zone B	Zone C	Zone A	Zone B	Zone C
C16: 0	4.30±0.01	3.89±0.02	5.11±0.03	4.14±0.04	3.94±0.02	4.98±0.04
C18:0	42.20±0.3	41.87±0.01	41.90±0.01	41.91±0.02	42.74±0.01	41.83±0.03
C18:1	45.20±0.04	46.12±0.03	45.30±0.01	45.72±0.01	45.09±0.01	45.10±0.02
C18:2	6.20±0.05	5.81±0.04	5.8±0.03	6.01±0.02	5.60±0.02	5.68±0.03
C18:3	0.83±0.05	0.94±0.04	0.69±0.07	0.81±0.03	0.88±0.05	0.74±0.07
C20:0	0.80±0.03	0.84±0.03	0.61±0.05	0.85±0.04	0.67±0.04	0.59±0.05
Total	99.53	99.47	99.41	99.44	99.00	98.92
ANOVA	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

0.50±0.03 to 1.34±0.02 and 0.55±0.04 to 1.26±0.03 for zones A, B and C respectively. Comparison of the respective mean dirt contents for the three zones and the two years span of this study showed no statistical difference (P<0.05) however, there was a general reduction in the average dirt content in the second year (Table 3). Zone A value was reduced from 1.36±0.28 to 0.94±0.26, zone B from 1.28±0.26 to 1.05±0.21 and zone C from 1.36±0.30 to 0.95±0.24. This suggests an improvement in the filtration, clarifying and other ancillary dirt removal procedures along the processing steps (skimming, decanting and scooping) among the zones. Dirt is an important parameter in quality consideration, for example, a butter refiner or end user will lose about 1.36 metric tonnes as dirt for every 100 metric tonnes of butter purchased from zone A. This is in addition to the uncalculated envisaged loss during re-filtration or re-processing. The high content of dirt in the butter, one of the hallmarks of low quality, could be attributed to foreign materials (debris, dust, sand etc.) sticking to the kernels or entering through dirty processing utensils. Strictly speaking, simple improvements in the overall processing practices of butter are capable of minimizing the amount of dirt while ensuring improved consistency in the butter quality.

Density

The result of density determination generally fall outside the 0.858 and 0.893 range for solid fat but there was significant difference (P< 0.05) among the zones in the first year but no significant difference was observed in the second year of sampling (Table 4). Among the villages, no significant difference was observed save in zones A and C in the first year, and zone B in the second year.

Melting range

The melting range was within the results of earlier researchers like Olaniyan and Oje (2007). The wider melting range observed for the respective villages could be attributed to the butter handling such as melting and re-melting the butter several times.

Moisture

The moisture contents of the butter from the villages in the respective zones were significantly different (p<0.05) both in the first and second year (Tables 1a, b, c and Tables 2a, b, c) results. The lowest and highest moisture contents were found in zone A (0.04±0.00, 0.52±0.03), zone B (0.08±0.01, 0.50±0.03) and zone C (0.09±0.01, 0.50±0.03) in the first year, and A (0.36±0.02, 0.53±0.01), B (0.36±0.02, 0.64±0.01) and C (0.21±0.02, 0.72±0.02) respectively. The mean percentage moisture content (Tables 3 and 4) for zone were: (0.26±0.23) for A and 0.36±0.12 for C (0.38±0.17) for the first year and 0.43±0.07 for A and 0.57±0.13 for B. The second year exhibited significant difference (p<0.05). The percentage moisture obtained in this study fitted into the third grade butter according to UEMOA standards (2006) (0.3-2.0) for unrefined shea butter. This indicates that there is need to improve the drying methods of the processing as well as proper storage system to minimize the moisture content hence upgrading the butter quality. It also shows that butter from zone C is more susceptible to hydrolytic reactions leading to shorter shelf life and high loss to a refiner.

Free fatty acid (FFA)

Free fatty acid (FFA) content of oil or fat offers a simply calculated index of quality, representing in effect the proportion lost to hydrolytic degradation. According to Masters et al. (2004), refining shea butter of 1% FFA will result in a loss of 1% of the original volume of the unprocessed butter. Statistical analysis of the first year data for FFA reveals a significant difference (P<0.05) (Table 1a, b and c). In the first year result of this study,

(Tables 1a, b and c), the percentage FFA of zone A ranged from 5.32±0.25 to 9.87±0.178 with an average of 8.03±1.76; zone B ranged from 6.71±0.22 to 13.60±0.02 with an average of 10.39±2.49 while in zone C, the range was 5.28±0.12 to 12.12±0.03 with an average of 9.63±2.10. In the second year (Tables 2a, b and 2c) zone A ranged from 7.32±0.09 to 9.67±0.03 and averaged 8.58±1.04: zone B ranged 4.95±0.06 to 10.13 ±0.06 and averaged 7.78±1.53 while the range in zone C was 4.62±0.03 to 8.74± 0.02 with an average of 7.03±1.51. The lowest FFA percentage of 4.62±0.03 was recorded in Dusai village in zone C in the second year data while the overall highest percentage of 13.60±0.06 was found in Gurmana village of zone B in the first year data. These values recorded in this study were similar to the values reported by other researchers who have worked on Nigeria shea butter (Olaniyan and Oje, 2007). However, the values were well outside the range of even the third grade of the UEMOA standard (2006). When the averages (significantly different($P<0.05$) for the zones, were considered for the two years, except for zone A that depreciated in quality, from 8.03 to 8.58 the other two zones appreciated [zone B(10.39 to 7.78) and zone C (9.63 to 7.03)]. This suggests that there were hydrolytic inducing activities along the collection processing and butter storage chain was employed by these set of processors. Shea butter of this range of quality will not attract premium price in the international market hence cannot adequately improve family income and compensate for the drudgery undergone by the processors. This result depicted the range of FFA content (which is generally outside those international market) of shea butter meant to be sold in various markets in Bida, Niger State.

In the case of Acid Value (AV) a statistical interaction was also observed between villages and zones for the quality of shea butter. Statistical analysis demonstrated a significant difference among the entire villages, 3 zones and for the two years. The discussions here followed the trend under FFA section, except that the values of AV were 1.99 multiples of FFA. This is also a clear indication of low quality shea butter.

Peroxide Value (PV)

Peroxides are the intermediate compounds formed during oxidation of lipids which may react further to form the compounds that can cause rancidity while PV determines the extent of fat or oil oxidation by measuring the amount of peroxides present in the oil or fat samples(AOCS, 2009). PV is another vital quality index. The values of this parameter ranged for the first year from 4.29±0.02 to 10.20±0.01 in zone A, 4.67±0.04 to 11.17±0.06 in zone B and 3.50±0.02 to 10.22±0.03 in zone C while in the second year, the ranges were 3.70±0.05 to 6.36±0.07, 3.79±0.04 to 7.31±0.02 and 2.74± 0.01 to 8.07±0.08 correspondingly. The magnitude of the PV exhibited by these butter samples from the respective zones were significantly different ($P<0.05$). When the mean values (Tables 3 and 4) were considered for the zones and the two years, significant differences existed, in that order, in the two data sets. In the second year, there was a discernable reduction in the mean values, zone A moved from 6.00±1.82 to 4.69±0.84, B from 7.83±1.93 to 5.39±1.36 C from 7.34±1.97 to 4.47±1.54. The generally low values recorded in the second year compared to the first year suggest minimum mixing of freshly processed butter with previously stored butter or an improvement in the processing methods or an increase in the secondary oxidation processes. These values were however within the UNBS standards of 10 milliequivalent oxygen per kilogram oil but about 3-4 times lower than the lowest value of 22.1 reported by Olaniyan and Oje (2007) for Nigerian shea butter. The far reaching implications of these discrepancies in the quality characteristics of Nigeria butter lend credence to the ad hoc and disjointed nature of the researches. Hence, the provision of the history of the kernel used by the various researchers for the experiment is vital in authenticating and, placing and referencing the results of their studies under appropriate categories of data. The result of the oxidation of fat and oil is the development of unpleasant flavours and odours characteristic of the condition known as oxidative rancidity. It has been found that oxidative abused fat can complicate nutritional and biochemical studies in animals because they can affect food consumption under *ad libitum* feeding conditions and reduce the vitamin content of the food. If the diet has become unpalatable due to excessive oxidation of the fat component and is not accepted by the animal, a lack of growth by the animal could be due to its unwillingness to consume the diet. Thus, the experimental result might be attributed unwittingly to type of fat or other nutrient being studied rather than to the condition of the ration (AOCS, 2009). Knowing the oxidative condition of unsaturated fats is extremely important in biochemical and nutritional studies with animals (AOCS, 2009).

Anisidine value (AnV)

AnV is another quality parameter that measures the secondary oxidation of fats and oil. The values of this index recorded in this study were significantly different in all the villages of the zones. Whereas the mean values for the zones in the first year were not significant different, they were in the second year. However, when the means of the two years (Tables 3 and 4) were compared, there was significant difference and this signified inconsistency in the quality. The lowest and highest value of 2.94±0.02 and 4.64±0.07 were recorded in village 4 and 5 of zone A respectively. This suggests that the butter samples have undergone through varying degrees of secondary oxidative degradation.

Iodine value (IV)

Fats and oils are made up of triglyceride molecules which may be saturated and unsaturated fatty acids. The degree of unsaturation of a fat, in other words, the number of double bonds present is normally expressed in terms of iodine value of the fat. The iodine value which is an identity parameter rather than quality is the number of grammes of iodine which will react with the double bonds in 100 grammes of fat or oil (AOCS, 2009). In this work, significant difference among the iodine values of butter was observed between villages in the respective zones. There was also significant difference in the mean values of the zones and for the two years study periods (Tables 3 and 4). In zone A and for the first year, the lowest IV of 38.67±0.68 was recorded in Koriagi village while the highest value of 55.77±0.35 in Gbangban; in zone B it was 41.20±0.30 (Gwada) and 60.37±0.57 (Zumba Shiroro) and zone C, 39.63±0.67 (Gulbin boka) and 58.63±0.38 (Borgu) in year two, (Tables 2a, b, c) the ranges for the zones were 39.43±0.6 to 50.83±1.02 for A, 36.97±0.21 to 52.57±0.25 for B and 39.03±045 to 60.57±0.42 for C. These variations in the values for the respective villages, zones and year were significantly different ($p< 0.05$) and similar to the value obtained by other researchers however, the values of Olaniyan and Oje, (2007) were higher. The variations observed in this work could be attributable partly to the intrinsic genetic variation of shea tree and partly due to the processing method particularly when inconsistent poor clarification methods that promote partial fractionation are used. Some of the local processors, in a bid to clarify the butter, allow the freshly produced butter to cool and solidify so that the dirt will be at the bottom of the container. Thereafter, they will scoop the upper layer leaving the predominantly dirty bottom with some portions of high melting saturated fraction. In other words, this implies that the partial inadvertent fractionation has altered the compositions of the said butter sample scooped in favour of the unsaturated fraction.

Saponification value

The saponification value is defined as the amount of potassium hydroxide (KOH) in milligrams required to saponify 1 g of fat or oil under the conditions specified. Based on the length of the fatty acids present in the triacylglycerol molecule, the weight of the triacylglycerol molecule changes which in turn affects the amount of KOH required to saponify the molecule. Hence, saponification value is a measure of the average molecular weight or the chain length of the fatty acids present. As most of the mass of a triglyceride is in the three fatty acids, it allows for comparison of the average fatty acid chain length. As seen from Tables 1a, b, c and 2a, b, c, the saponification value for the majority of the butter samples are in the range of 189.83 to 236 mg KOH/g found respectively in villages 5 and 1 during the first year of this study. This range is typical for fats having predominately fatty acids with a long chain between C16 and C18 because higher saponification values may indicate the presence of shorter chain lengths. The SV values obtained in this study is generally below the values of 237.7 to 261.3 mg KOH/g reported by Olaniyan and Oje, (2007), while some of them fell within the UNBS standards (2004) range 170-190 mgKOH/g.The variation in the SV values may be due to processing, fruit harvesting and kernel storage methods (Lovett, 2004) as well as integrity of the kernels processed and other additives like anti-oxidants.

Unsaponifiable matter (UM)

In general, unsaponifiable matter content of edible oils is about 2% and they include tocopherols/tocotrienols, other phenolics, phytosterols, hydrocarbons, among others (Di Vincenzo et al, 2005; Esuoso et al, 2000). The content of the native unsaponifiable matters varies in different oils and, depends on the extent and methods of oil refining. In this study, there was significant difference in the unsaponifiable matter contents of the butter from Bida. The overall highest and lowest percentage values of 10.09 were obtained in villages 6 in the second year and 5.93 in village 8 in the first year. The differences observed in this study may be due to a combined effect of processing methods used and the highly inherent tree-to-tree variations found in shea tree IPGRI, 2010). Due to the high unsaponifiable matter contents (Table 4) of butter from Bida, Nigeria, the cosmetic industries will prefer to buy shea butter from Bida.

Fatty acid composition

In this study, five major fatty acids were identified which is consistent in similar proportion with the result of other researchers (Okullo et al., 2010; Nahm, 2011). Although there were significant differences among and within the zones, the characteristics relative abundance of the five major fatty acids was not altered. The C18:1 and C18:0 consistently remained the most abundant unsaturated and saturated fatty acids respectively. This also shows no adulteration with other oils or fats.

Conclusion

Overall, this study provides current baseline data on shea butter intended for sale in Niger State Nigeria. The study reveals that the butter quality is low and inconsistently varies from village to village and from zone to zone. The quality of the butter satisfies the local segment of the market because a refiner or other end users will definitely

incur irrecoverable loss due to high contents of free fatty acid, dirt, and moisture besides other refining challenges. Besides, initiating and providing a current data bank on shea butter quality in Nigeria has revealed those factors (high dirt, FFA, moisture, peroxide contents) that inhibit Nigeria shea butter from commanding a premium price in the international market. The marginal improvement in the quality parameters in the second year showed that the local processors have capacity for producing high quality butter of international standards.

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Full Length Research Paper

Effect of heat treatments on sensory characteristics and shelf-life of skimmed milk

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The present study was conducted to observe the effect of various heat treatments on sensory characteristics and shelf-life of skimmed milk at the Department of Animal Products Technology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam, during year 2012-2013. The skimmed milk was produced from buffalo milk through cream separator and then divided into four groups viz. A, B, C and D. The group A was kept as untreated (control) and B, C, D (treated at 60°C for few s (thermization), 65°C for 30 min (pasteurization), 110°C for 10 min (sterilization), respectively. The parameters studied included; sensory attributes such as appearance/color, taste/flavor and body/texture scores and shelf-life at cold storage (6±2°C) and at room temperature. Appearance/color of pasteurized and sterilized skimmed milk was not acceptable compared to thermizide and control skimmed milk. Sterilization process improved the taste/flavor and body/texture of skimmed milk. Shelf-life of skimmed milk was remarkably (P<0.05) better with sterilization process at room temperature as well as at cold storage (6±2°C).

Key words: Heat treatment, sensory characteristics, skimmed milk.

INTRODUCTION

Milk is a perishable and complete food, used as the primary source of nutrition for humans as well as other mammals. It is used as whole milk or as milk products including cheese, yoghurt, butter, ice cream etc. The exact components of raw milk vary by species. In addition to cattle, the milk of buffalo, goat, sheep and yak is used for the manufacture of dairy products. Historical evidence shows that the nations which used the milk and its products were found to be more civilized and capable of having sound administration (Athar et al., 2003). Skimmed milk is known as reduced fat milk obtained by skimming of milk through cream separator. The primary objective to make skimmed milk is to reduce its fat contents thus it can be easily utilized by everyone (Sangwan, 2008). Skimmed milk is used in many food products like cheese, flavored beverages, cakes and breads, etc. On various food

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products, it does not impart only milky flavor, but also desirable properties to satisfy the consumer preference. Richness of skimmed milk with lactose, proteins, calcium etc, increases the nutritional value of the products. It is also an excellent alternative for those peoples who are conscious of taking fat in their diets (Mulvihill and Ennis, 2003). Heating is always employed to ensure microbiological safety, but in cases where milk and its products are used as food ingredients, heat treatment is employed to improve the organoleptic properties of such dairy formulations by manipulating the functionality of milk proteins (Del Angel and Dalgleish, 2006). Undesirable changes in dairy products may be investigated by microbial growth and metabolism or by chemical reactions. The spoilage microbes may induces undesirable changes in the taste and odor of milk such as sour, putrid, bitter, malty, fruity, rancid and unclean. The processors preserve and process the milk by thermal processing to meet the increasing demand of milk round the year in the markets. Various thermal processing techniques are being applied in the dairy industry, carried out by subjecting milk at different temperature and time combinations in order to have a long shelf-life at room temperature (Valero et al., 2001). Sensory evaluation is regarded as a test for evaluation as well as acceptance of milk and its products by consumers. It cannot determine the quantity of various components of the product but the products are evaluated through color, consistency, texture and odor etc. Thus sensory evaluation helps to provide the processors and producers with a guide to consumer acceptance for the products (Phillips et al., 1995; Saba et al., 1998). The present study has been planned to evaluate the influence of pasteurization, sterilization and thermization on the sensory characteristics as well as shelf-life of treated skimmed milk.

MATERIALS AND METHODS

Milk sample

Buffalo milk obtained from Livestock Experiment Station, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam was used during the present investigation.

Equipments/utensils

Hot air oven

Hot air oven (Memmert 854, Schawabch W. Germany) was used for sterilization of equipments/ utensils during research work.

Analytical balance

Analytical balance (Adam, Model No. AAA 2502) was used to take weight of samples and chemicals during analysis.

Gerber centrifuge machine

Centrifuge machine (Funke Gerber, Model No. 12105 Germany) was used to centrifuge the samples during determination of fat content of heat treated and untreated (control) skimmed milk samples.

Cream separator

Cream separator (Domo, Sweden) was used for skimming or separation of cream from whole buffalo milk.

Water bath

Water bath was used to process the skimmed milk samples for thermization.

Refrigerator

Refrigerator (Dawlance, 9188wbm, Pakistan) was used to store the milk samples during determination of shelf-life of the heat treated and untreated (control) skimmed milk samples.

Experimental procedure

The present study was conducted to observe the influence of various heat treatments on sensory quality and shelf-life of skimmed milk. The whole buffalo milk (3 L) collected from Livestock Experiment Station, Department of Livestock Management, was brought to the Dairy processing laboratory, Department of Animal Products Technology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam. As soon as the milk was received, it was filtered through muslin cloth and volume was measured. Then, it was pre-heated to 40°C and transferred to the supply tank of cream separator for skimming. After skimming, the milk was measured into four equal parts and accredited with A, B, C and D codes. The samples of skimmed milk coded with B, C and D were heated at temperatures of 60°C for few s (Thermization), 65°C for 30 min (Pasteurization) and 110°C for 10 min (Sterilization), respectively while sample coded with A was kept as non-heated (Control) for comparison purpose. A total of six trials each in duplicate batches were conducted and analyzed for sensory characteristics such as appearance/color, taste/flavor and body/texture scores and shelf-life at cold storage (6±2°C) and at room temperature.

Analysis of sensory characteristics

Sensory characteristics of thermizide, pasteurized, sterilized and un-treated (control) skimmed milk was analyzed according to the method as reported by Nelson and Trout (1981). A panel of five judges was selected from M.Phil. students of Department of Animal Products Technology. The judges were first familiarized with sensory profile of all the samples. Thereafter, the samples were offered for evaluation. The score was rated on hedonic scale of 10 for appearance/color, 45 for taste/flavor and 30 for body/texture.

Analysis of shelf-life

Shelf-life of each sample was obtained using clot on boiling (COB) test. 5 ml of each heat treated and untreated (control) skimmed milk samples were taken in screw capped test tubes and heated for 5 min. In case of any curd formation or precipitation noticed the sample was considered as expired.

Statistical analysis

The data was analyzed according to statistical procedure of analysis of variance (ANOVA), and in case of significant differences, the mean were further computed using least significant difference (LSD) at 5% level of probability through computerized statistical package that is Student Edition of Statistix (SXW), version 8.1 (Copyright 2005, Analytical software, USA).

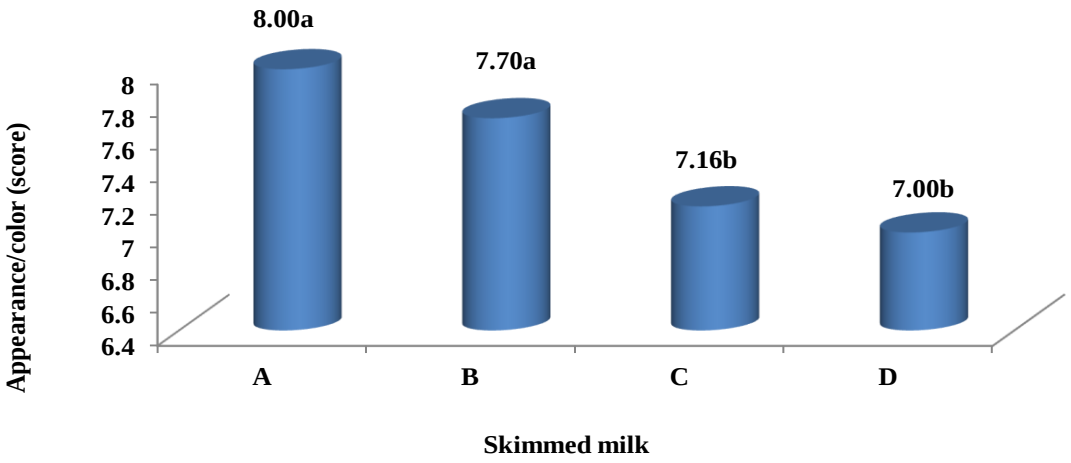

Figure 1. Appearance/color (score) of thermizide, pasteurized, sterilized and control skimmed milk. LSD (0.05) = 0.3477; SE± = 0.1667.

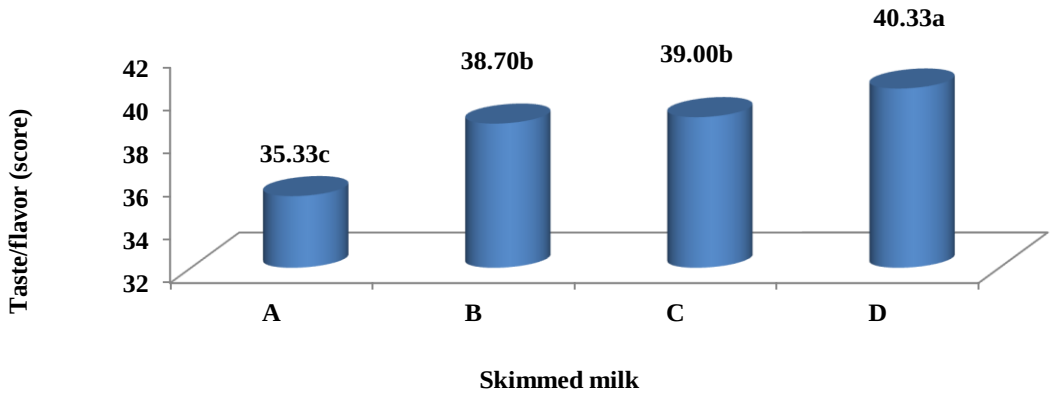

Figure 2. Taste/flavor (score) of thermizide, pasteurized, sterilized and control skimmed milk. LSD (0.05) = 1.3193; SE± = 0.6325.

RESULTS

Effect of heat treatments on sensory quality of skimmed milk

Appearance/color

The appearance/color score of heat treated and untreated (control) milk was evaluated by judges and score perceived is shown in Figure1. Results reveal that no remarkable (P>0.05) influence of thermization process was on appearance/color of skimmed milk. The score rated for thermizide skimmed milk (7.7) was not significantly different from that of control skimmed milk (8.00). Moreover, pasteurization and sterilization processes revealed statistically comparable (P<0.05) results. Regardless, the score rated for pasteurized and sterilized skimmed milk was relatively similar (P>0.05), and the score rated for untreated (control) skimmed milk was comparatively (P<0.05) higher than that of pasteurized and sterilized skimmed milk.

Taste/flavor

The taste/flavor perception scores of various heat treated and untreated (control) milk was evaluated by judges and score perceived is presented in Figure 2. The taste/flavor score was improved in heat treated skimmed milk compared to un-treated (control) skimmed milk [35 to 36 to untreated (control) skimmed milk samples]. It was noticed that the heat treated (thermizide, pasteurized and sterilized) skimmed milk samples received better taste/flavor scores (38.70±0.54, 39.00±0.63 and 40.33±0.33, respectively) compared to that of un-treated skimmed milk (35.33±0.21). Moreover, LSD (0.05) comparison of means revealed that thermization and pasteurization processes has similar influence (P>0.05) on taste/flavor score of skimmed milk, while sterilization process perceived better score for taste/flavor among both these processes.

Body/texture

The body/texture scores of heat treated and un-treated

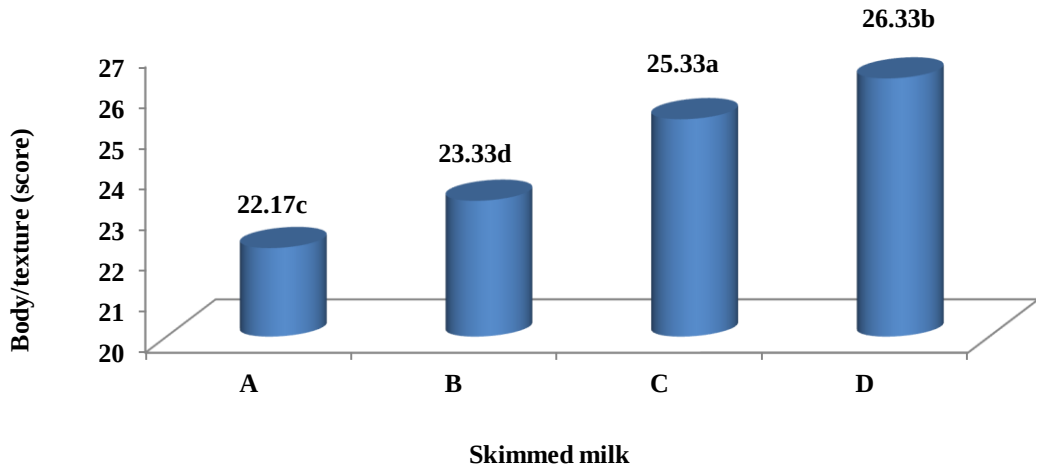

Figure 3. Body/texture (score) of thermizide, pasteurized, sterilized and control skimmed milk. LSD (0.05) = 0.8004; SE± = 0.3837.

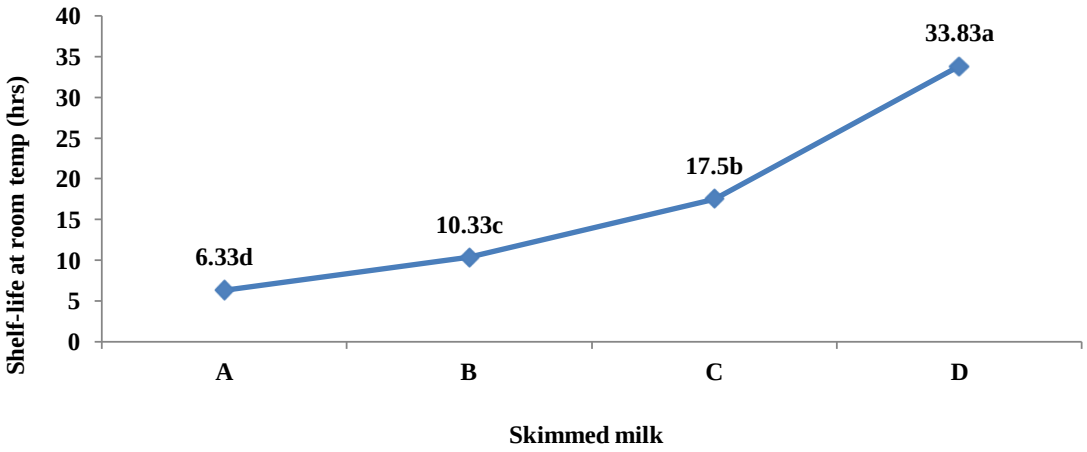

Figure 4. Shelf-life of thermizide, pasteurized, sterilized and control skimmed milk at 30°C. LSD (0.05) = 0.7125; SE±; = 0.3416.

(control) milk was evaluated by judges and the rated score is shown in Figure 3. The body/texture score was improved with thermization, pasteurization and sterilization processes. Control skimmed milk received an average score (22.17±0.17) from total of 30. The score was enhanced to 23.33±0.33 in thermized skimmed milk, 25.33±0.21 in pasteurized and 26.33±0.33 in sterilized skimmed milk samples.

Shelf-life of treated and untreated skim milk at room temperature and 6±2°C

The shelf-life of thermizide, pasteurized, sterilized and un-treated (control) milk samples stored at room temperature and cold storage (6±2°C) was evaluated through clot on boiling (COB) test, and the results are presented in Figure 4. It was found that un-treated (control) skimmed milk samples gave positive results on COB within 6 h of storage at room temperature and 1 day (24 h) at cold storage (6±2°C) temperature. Similarly, the average shelf-life was 11 h at room temperature and 3 days on cold storage (6±2°C) temperatures, for thermizide skimmed milk samples, 18 h at room temperature and 12 days at cold storage (6±2°C) temperatures, for pasteurized skimmed milk samples and 33 h at room temperature and 28 days at cold storage (6±2°C) temperatures, for sterilized skimmed milk samples.

DISCUSSION

The shelf-life of untreated (control), heat treated skimmed milk samples were evaluated through COB under room and cold storage (6±2°C) temperatures. It was concluded that shelf-life of skimmed milk was significantly less than that of whole milk treated at various pasteurization temperatures. The present results are in favor of the findings

by Janzen et al. (1982), who observed that skimmed milk has significantly less shelf-life than whole milk because of greater proteolysis in skimmed milk as compared to whole milk, thus resulted to increase in the production of protease enzyme which increases the susceptibility of protein to protease attack thus decreases the shelf-life of the treated skimmed milk samples.

The overall acceptability was increased with increase temperature in treated skimmed milk samples as compared to untreated samples in the present study. These findings are in contrast to the results of Hussain (2011), who reported that changes in temperature adversely affected the scores for taste, appearance and body of skimmed milk. He further reported that the changes in organoleptic properties of skimmed milk could occur due to proteolysis process, lactose degradation, millard reaction, etc. which is much faster in samples treated above 100°C and the results in changing the color and flavor of milk samples. Present results are also in cross with the findings by Petrus et al. (2011), who concluded that temperatures ranged from 72 to 94°C/ 15 s has no effects on sensory characteristics of milk.

In the present study, the skimmed milk was prepared from whole buffalo milk at the Department of Animal Products Technology, Sindh Agriculture University Tandojam, during year 2012-2013, and processed for thermization (60°C for few s), pasteurization (65°C for 30 min) and sterilization (110°C for 10 min) processes. All the heat treated skimmed milk samples were examined against the control (un-treated) skimmed milk for physico-chemical (pH, titratable acidity, and specific gravity, moisture, fat, protein, lactose and ash contents), sensory attributes (appearance/color, taste/flavor and body/texture) and shelf-life.

Perception score rated for the appearance/color of the pasteurized and sterilized skimmed milk was relatively similar ($P>0.05$), but control skimmed milk was rated comparatively ($P<0.05$) higher score from that of all treated skimmed milk samples. The score rated for taste/flavor was found to be higher in sterilized skimmed milk in comparison to all other samples whereas, thermization and pasteurization processes showed similar ($P>0.05$) influence on taste/flavor of skimmed milk. Body/texture of skimmed milk was improved as temperature was increased. The score rated for sterilized milk was significantly ($P<0.05$) higher, followed by pasteurized, thermizide and control skimmed milk samples. The shelf-life of sterilized skimmed milk under room temperature and cold storage (6±2°C) was significantly ($P<0.05$) higher followed by pasteurized, thermizide and control skimmed milk.

Conclusion

The taste/flavor and shelf-life of skimmed milk were remarkably improved with the use of sterilization process.

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Full Length Research Paper

Detection of proteolysis in high temperatures treated milk by Reverse phase high performance liquid chromatography (RP-HPLC)

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Proteolysis of UHT milk during storage is one of the problems affecting the dairy industry worldwide. Native enzymes have been implicated as being the main cause of spoilage of such milk. In the current study, reverse phase high pressure chromatography (RP-HPLC) method was used to detect proteolysis by native enzymes in high temperature heated milk. The aim of this research was to assess susceptibility of milk to proteolysis by native enzymes, after being subjected to various temperature-time processing conditions. Samples of raw and heated milk were clarified prior to analysis. Clarification was carried out to obtain pH 4.6 and 6% TCA soluble extracts, which were injected into RP-HPLC after filtration. This method confirmed that raw milk and milk processed at 85°C /15 s were the most proteolysed, indicating that the high temperatures employed during this study (110, 120, 130 and 142°C for 2s) inactivated the native enzymes. The RP-HPLC method is a useful method for the detection of proteolysis in milk.

Key words: Reverse phase-high performance chromatography (RP-HPLC), proteolysis, milk, plasmin, heat-treatments.

INTRODUCTION

Heat treatment of milk is used for pasteurisation/sterilization and to promote desirable physical characteristics in the protein or food system (Raikos, 2010). During heat treatment, chemical, physical and biochemical reactions take place. These changes are significant because they influence nutritional, sensorial and microbiological aspects of milk. The reactions include Maillard reactions, denaturation and aggregation of whey protein, and formation of complexes between whey proteins, caseins and fat globules (Corredig and Dalgleish, 1999).

Milk contains various proteolytic enzymes which degrade milk proteins. These proteases are either secreted into the the milk during milk synthesis or originate from bacteria (Fox and McSweeney, 2003). Normally, psychotrophic bacteria would not be a serious problem in heat treated milk, but their proteases survive UHT treatment and are readily able to degrade α_{s1}, α_{s2}, β and κ-caseins, causing proteolysis (Sorhaug and Stepaniak, 1997), and furthermore these heat-stable proteases can affect the PL system. Bovine milk contains several proteases including plasmin, plasminogen, plasminogen activators, thrombin, cathepsin D, acid milk proteases and aminopeptidase (Fox and Kelly, 2006). Plasmin (EC 3.4.21.7), occurs in the highest amount and is one of the most heat resistant

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enzymes in milk (Datta and Deeth, 2003). It is part of a complex system consisting of plasminogen (PG), plasminogen activators (PA), plasminogen activator inhibitors (PAI), plasmin (PL) and plasmin inhibitors (PI) (Crudden and Kelly, 2003). It is a heat resistant enzyme with optimum activity at 37°C and pH 7.4 and hydrolyses mainly β- and α_{s2}-caseins, and more slowly α_{s1}-casein (Bastian and Brown, 1996). The rate and extent of proteolysis is determined by the types and activities of the proteolytic enzymes present (Pereda et al., 2008). These proteases have been studied from the perspective of their physiological significance in milk, their effect on the processing of dairy products, and their nutritional and antimicrobial roles (Aslam and Hurley, 1998). Proteolytic enzymes are of great importance to the dairy industry because they are responsible for imparting desirable or undesirable properties to dairy products through changes in flavour and texture (Nilsen, 2002).

The aim of the current study was to investigate susceptibility of milk processed at various temperature-time profiles to proteolysis by native enzymes during storage, with raw milk as a control. The study was also aimed at observing changes (if any) in protein breakdown products by reverse phase high pressure liquid performance (RP-HPLC).

MATERIALS AND METHODS

Unless otherwise stated, all materials were from Fisher (Fisher Scientific UK Ltd, Leicestershire, UK). Raw milk was obtained from the Centre of Dairy Research (CEDAR), University of Reading, UK. It was processed on an APV junior UHT plate heat exchanger (APV, Crawley, UK), with two stages of heating involving hot water (80°C) and steam (112-142°C) as described (Browning et al., 2001). A constant flow rate was used, giving a residence time of 2 s in the holding section at 110, 120, 130 and 142°C but 15 s at 85°C. Homogenisation took place between the heating stages at about 170 bars. These temperature-time combinations were selected based on studies of plasmin inactivation. The lowest (85°C/ 15 s) was chosen so as to mimic pasteurisation, whereas others were in a range where inactivation of plasmin could occur and therefore it would be interesting to monitor changes in proteolysis with time.

After cooling, the samples were stored at 2°C for 2 days. The six batches of milk samples were treated with sodium azide (0.05%) to prevent bacterial contamination. They were then dispensed in sterile bottles in a laminar flow hood cabinet followed by incubation at 37°C for 28 days. Sampling for analysis was done on days 0, 3, 7, 14, and 28. Clarification to obtain pH 4.6 and 6% TCA soluble extracts was carried out as detailed below.

Clarification procedures were carried out after incubation for all milk samples studied. Prior to clarification, all milk samples were heated and held at 100°C for 10 min to denature the whey proteins. For isoelectric precipitation at pH 4.6, 50 mL warm water (40°C) was added to 5 mL of milk followed by 0.5 mL 10% (w/v) acetic acid. After standing for 10 min, 0.5 mL 1 M sodium acetate was added and placed in cold water for 10 min before filtration through Whatman no. 41 filter paper and washing and making up to 100 mL. The clear extracts obtained were further filtered by 0.2 µm millipore filter before being subjected to the RP-HPLC methods.

To obtain 6% TCA soluble extracts, 5 mL of 12% (w/v) trichloroacetic acid (TCA) was added to an equal volume of milk (raw, pasteurised/ UHT). The test tubes were vortexed for 2-3 min and left at room temperature for 1 h. The solutions were vortexed again for 2-3 min followed by filtration through Whatman no 41. The filter paper was washed with water and the volume of the supernatant made up to 10 mL with distilled water. Filtration was further carried out by 0.20 µm Millipore filter.

Analysis by RP-HPLC was performed on Dionex chromeleon equipment consisting of a P580 pump (Dionex Corporation, Munich, Germany) with a photodiode array detector (Dionex PDA-100), an automated sample injector and a C18, 5 µm, 80Å, 150 x 4 mm STH 585 version 2.5 HPLC column compartment with reverse phase column (SGE 150 GL4-C-P-8/5, Melbourne, Australia) at 40°C. Data analysis was computed by the Chromeleon Datasystem software v. 6.50 SP4 Build 1000. The flow rate was 0.75 mL/min and detection was by a UV/Vis detector at 210 nm. Solvent A was 0.1% (v/v) trifluoroacetic acid (TFA) in HPLC grade water whereas Solvent B was 0.1% (v/v) TFA in HPLC-grade acetonitrile. The volume injected was 50 µL. During the first 25 min, solvent B was increased from 15 to 35%. After 5 min, it was increased to 100% solvent B and held for 10 min. The column was then equilibrated at 15% solvent B for 10 min in readiness for the next sample injection. The peptides were quantified by integration of peak areas and expressed as the sum of peak areas.

Statistical analysis was carried out by using Statistical Package for Social Sciences (SPSS version 16). General Linear Model of analysis of variance (ANOVA) was used to determine statistical differences between means. Least square differences LSD (LSD) and Duncan's multiple range tests were used to determine values that were statistically different (P <0.05).

All analyses were carried out in triplicate, and results are expressed as mean ± standard deviation (SD).

RESULTS

pH 4.6 soluble extracts

Proteolysis increased with storage time and differed among samples (Table 1). From day 0 to 28 of incubation, pH 4.6 soluble extracts of milk heated at 85°C had the highest peak area which was statistically different ($p < 0.05$) from all other samples. The second highest peak was from raw milk, followed by milk heated at 110°C/ 2s and finally the last three milk samples had more or less similar peak areas over the storage period (Table 1).

Significant statistical differences in proteolysis ($p < 0.05$) were observed for raw milk, samples heated at 85 and at 110°C on each day of analysis. Figures 1 and 2 show chromatograms of pH 4.6 soluble extracts of raw milk and milk processed at high temperatures which had been incubated at 37°C for 7, 14 and 28 days. From each of these chromatograms, a high peak was observed at around 34 min for all samples, which had previously been linked to activity by plasmin.

From days 7 to 14, pH 4.6 soluble extracts of raw milk samples and of samples heated at 85, 110 and 120°C had higher peak areas which were statistically different ($p < 0.05$) from the rest of the samples. On day 28 however, only raw milk, samples heated at 85 and 110°C were significantly different in proteolysis from each other and

Table 1. Peak areas of pH 4.6 soluble extracts of raw milk and milk processed under various temperature – time conditions and incubated at 37°C for 28 days to examine the effect of proteolysis on storage time by the RP-HPLC.

Incubation time (days)	Treatments	Total peak areas of pH 4.6 soluble extracts of milk samples (m AU*min)
day 0	Raw milk	21.2 ±1.69 a A
	Heated at 85°C	23.7±2.20 b F
	Heated at 110°C	19.6±2.77 a K
	Heated at 120°C	20.9±1.75 a P
	Heated at 130°C	19.7±1.04 a T
	Heated at 142°C	20.8±1.68 a X
day 3	Raw milk	48.4±4.77 c B
	Heated at 85°C	50.5±8.18 c G
	Heated at 110°C	36.2±2.30 d L
	Heated at 120°C	23.6±0.92 e Q
	Heated at 130°C	23.2±2.36 e U
	Heated at 142°C	21.0±0.51 e X
day 7	Raw milk	57.6±2.77 f C
	Heated at 85°C	68.6±3.70 g H
	Heated at 110°C	40.8±1.94 h M
	Heated at 120°C	24.0±1.49 i G
	Heated at 130°C	20.8±2.03 j UV
	Heated at 142°C	21.6±0.52 ij X
day 14	Raw milk	70.3±7.21 k D
	Heated at 85°C	252.9±12.12 l I
	Heated at 110°C	44.3±4.07 m N
	Heated at 120°C	26.5±2.48 n R
	Heated at 130°C	23.2±1.13 n V
	Heated at 142°C	21.3±1.07 n X
day 28	Raw milk	212±8.15 o E
	Heated at 85°C	3159.43 p J
	Heated at 110°C	60.4±1.89 oQ
	Heated at 120°C	29.7±2.07 r S
	Heated at 130°C	25.8±2.78 rs W
	Heated at 142°C	22.7±2.57 s X

Different lower case letters on the same column show significant differences ($p<0.05$) per day whereas different uppercase letters on the same column show significant differences ($p<0.05$) per sample; The experiment was replicated 3 times (N=9); Error bars indicate standard deviations; The pH 4.6 soluble extracts were diluted (x20)

from the remaining samples (milk heated at 120, 130 and 142°C) which were not significantly different ($p>0.05$).

6% TCA soluble extracts

The 6% TCA soluble extracts indicated that although there were significant differences in proteolysis on day 0, the peak areas were generally low as indicated in Table 2. On day 3, statistical differences in proteolysis ($p<0.05$) were observed between the raw milk samples heated at 85 and at 110°C which had higher peak areas than samples processed at higher temperatures (120, 130 and 142°C).

From days 7 to 14, 6% TCA soluble extracts of raw milk samples and of samples heated at 85, 110 and 120°C had higher peak areas which were statistically different from the rest of the samples. On day 28 however, raw milk, samples heated at 85 and at 110°C were significantly different in proteolysis from each other and from the remain-

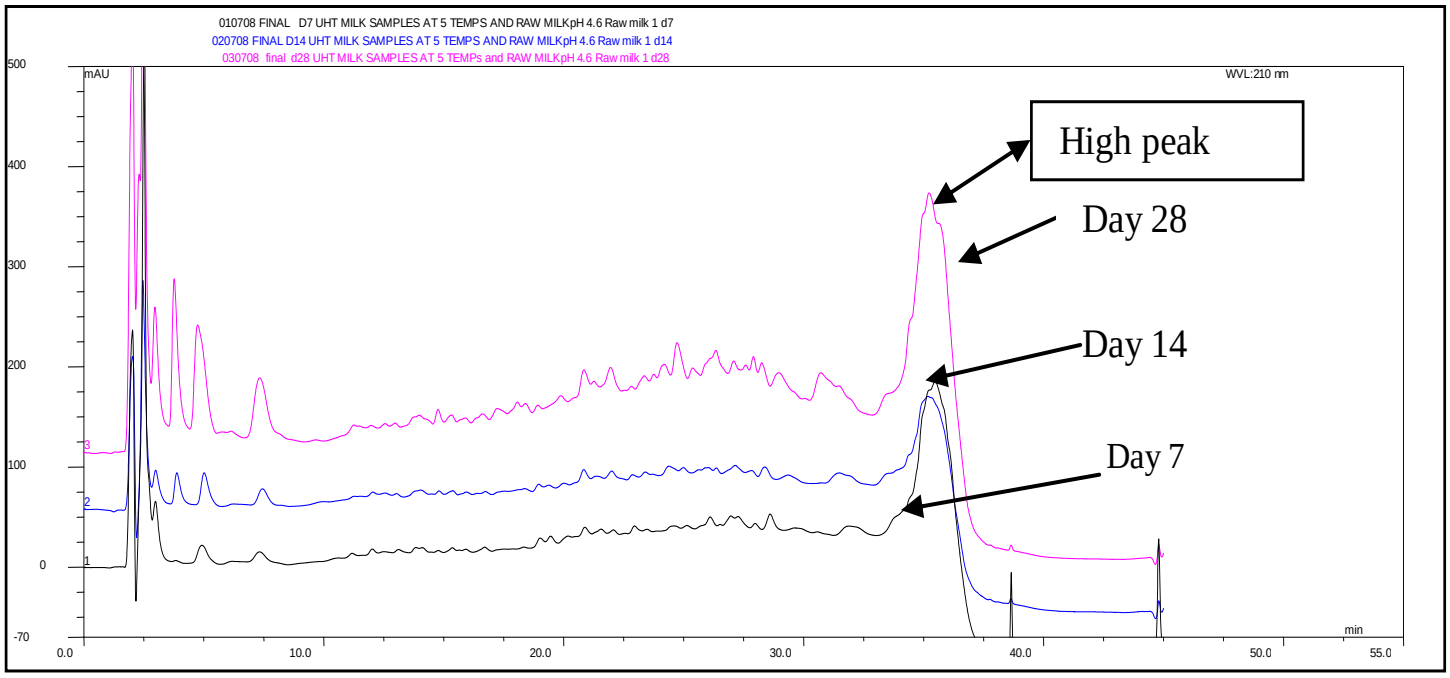

Figure 1. Sample chromatogram of pH 4.6 soluble extracts of raw milk incubated at 37°C for 7, 14 and 28 days.

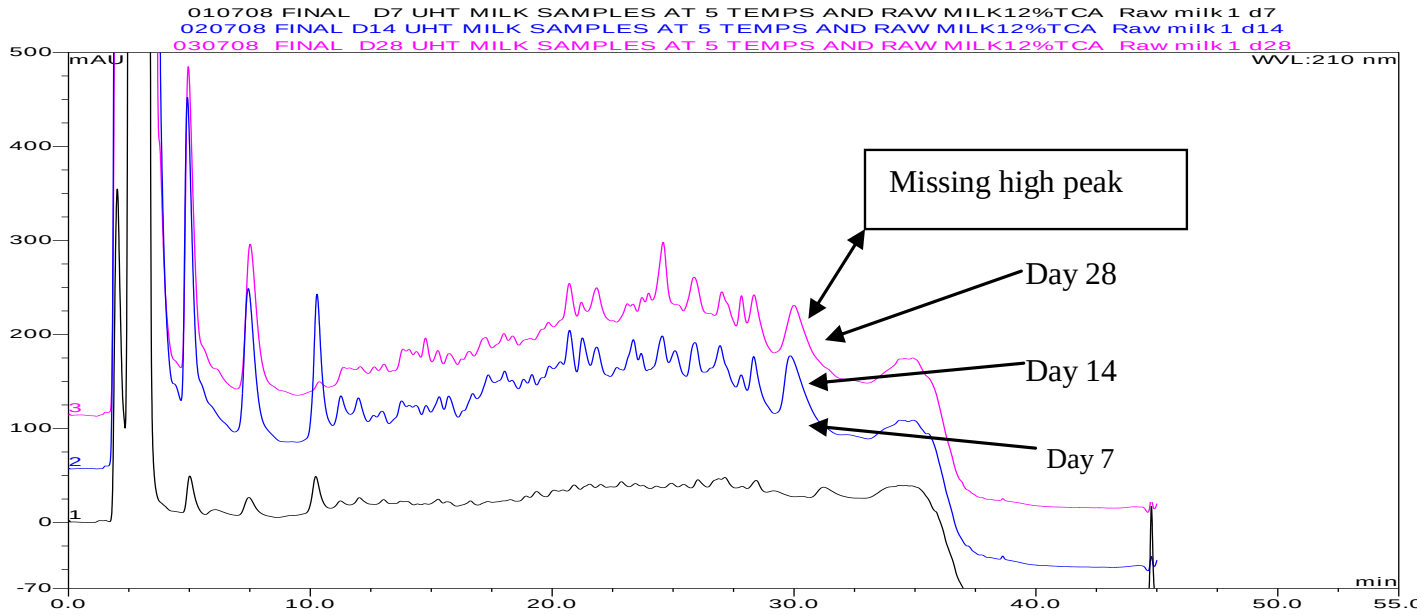

Figure 2. Sample chromatogram of 6% TCA soluble extracts of raw milk incubated at 37°C for 7, 14 and 28 days.

ing samples ($p < 0.05$).

Based on the day of incubation, it was evident that raw milk, milk heated at 85 and at 110°C shows significant differences ($p<0.05$) in proteolysis (Table 2). Proteolysis in the samples of milk heated at 130 and 142°C was low and varied from day to day. The 6% TCA soluble extracts of milk heated at 85°C had the highest activity than any other sample for all incubation days followed by 6% TCA soluble extracts of raw milk. The same trend was observed for pH 4.6 soluble extracts.

Table 2. Peak areas of 6% TCA soluble extracts of raw milk and milk processed under various temperature – time conditions and incubated at 37°C for 28 days to examine the effect of proteolysis on storage time by the RP-HPLC.

Incubation time (days)	Treatment	Total peak areas of 6% TCA soluble extracts of milk samples (m AU*min)
day 0	Raw milk	32.1±2.00 a A
	Heated at 85°C	33.9±1.25 b F
	Heated at 110°C	29.4±1.02 c K
	Heated at 120°C	29.2±1.25 c P
	Heated at 130°C	27.9±1.75 c U
	Heated at 142°C	25.9±1.57 d Y
day 3	Raw milk	125 ±5.01 e B
	Heated at 85°C	137±3.37 f G
	Heated at 110°C	51.9±3.50 g I
	Heated at 120°C	36.2±3.26 h Q
	Heated at 130°C	33.6±2.40 hi Y
	Heated at 142°C	29.8±1.72 i Y
day 7	Raw milk	134±3.89 j C
	Heated at 85°C	161±6.83 k H
	Heated at 110°C	60.7±3.43 l M
	Heated at 120°C	47.2±2.97 m R
	Heated at 130°C	39.9±3.93 n W
	Heated at 142°C	40.8±7.77 n Z
day 14	Raw milk	237±7.10 o D
	Heated at 85°C	442±6.04 p I
	Heated at 110°C	79.0±2.63 o N
	Heated at 120°C	60.0±7.29 r S
	Heated at 130°C	50.6±3.04 s X
	Heated at 142°C	49.0±2.85 s Z
day 28	Raw milk	330±9.61 t E
	Heated at 85°C	791 ±11.60 u J
	Heated at 110°C	90.9±7.93 v O
	Heated at 120°C	53.3±3.92 w T
	Heated at 130°C	50.2±3.72 w Z
	Heated at 142°C	49.8±2.85 w Z

Different lower case letters on the same column show significant differences ($p<0.05$) per day whereas different uppercase letters on the same column show significant differences ($p<0.05$) per sample; the experiment was replicated 3 times (N=9); Error bars indicate standard deviations; the 6% TCA soluble extracts were diluted (x2).

DISCUSSION

Effect of storage time on pH 4.6 soluble extracts

Based on the results shown in Table 1, it is apparent that significant levels of breakdown products were observed on each sampling day. Higher proteolytic products concentration observed in raw milk samples were probably due to the presence of the native enzymes, which resulted in proteolysis of the samples. However, the decrease in peak areas as temperatures increased especially at temperature higher than 110°C, is probably due to destruction of plasmin and plasminogen activator. This in turn, might have decreased plasmin activity on the caseins and hence decreased proteolysis. During severe heat treatments such as UHT processing, decreased plasmin activity is due to thiol-disulphide interactions between disulphide groups in plasmin and reactive SH groups of β-lactoglobulin during the unfolding and denaturation that occurs at high temperatures (Grufferty and Fox, 1986; Kelly and Foley, 1997). Other changes that were described in association with severe heat treatments include denaturation of

of whey proteins especially β-lactoglobulin, leading to the formation of β-lactoglobulin-κ-casein complex example through interaction with κ-casein (Datta and Deeth, 2003; McMahon, 1996).

As presented in chromatograms (Figure 1), a high peak was observed at around 34 min for all samples. The peak was highest in pH 4.6 soluble extracts from samples heated at 85°C followed by those from 110°C. It is documented that during pasteurisation, plasminogen activator inhibitors, being heat labile, are destroyed causing more plasminogen to be converted to plasmin resulting in increased proteolysis (Grufferty and Fox, 1988; Richardson, 1983). Several studies regarding the inactivation of plasmin have been documented. A study was reported where pasteurisation at 72°C for 15 s and 63°C for 30 min increased proteolytic activity by 30-40 and 8-24%, respectively, after incubating at 37°C for 3-6 days (Noomen, 1975). Increased proteolysis after pasteurisation was also reported (Andrews, 1983; Andrews and Alichanidis, 1983). Several authors have documented that pasteurisation destroys the plasminogen activator inhibitors and thereby more plasminogen is converted to plasmin by the plasminogen activators (Grufferty and Fox, 1988; Nielsen, 2000; Datta and Deeth, 2001; Richardson, 1983).

At higher temperatures however (>110°C), less activity was observed probably due to destruction of plasmin itself.

The peaks also revealed that the highest rates of proteolysis occurred in the pH 4.6 soluble extracts of milk heated at 85°C and of raw milk than any other sample.

Effect of storage time on 6% TCA soluble extracts

The first four milk samples in Table 1 shows significant differences ($p<0.05$) in proteolysis at each day of incubation. Proteolysis in the last two samples was low and varied from day to day. The 6% TCA soluble extracts of milk heated at 85°C had the highest activity than any other sample for all incubation days followed by 6% TCA soluble extracts of raw milk. This was also observed for pH 4.6 soluble extracts (Table 1).

The peak at 34 min which was apparent in all pH 4.6 soluble extracts was not so evident in 6% TCA soluble extracts samples (Figure 1). The current results are in agreement with the previous studies (Lopez-Fandino et al., 1993; Datta and Deeth, 2003)). The authors explained that unlike bacterial proteases which break down proteins into smaller peptides, plasmin forms large peptides which are precipitated by TCA and hence would not appear in 6% TCA soluble extracts, but in pH 4.6 soluble extracts. Higher peak areas were observed for 6% TCA soluble extracts of samples heated at 85°C followed by raw milk sample. All the other samples had quite low peak areas which progressively decreased with increasing heating temperature (Figure 1). Another study revealed that it was impossible to determine precipitation threshold in relation to peptide size as peptides containing 7-30 residues may be soluble, insoluble or partially soluble at the various TCA concentrations (Yvon et al., 1989). These authors suggested that interactions between TCA and the peptides induce an increase of the hydrophobicity of the peptides which leads to aggregation through hydrophobic interactions. It is likely that the larger peptides had been broken down during storage and leading to the formation of smaller peptides and amino acids which were soluble in TCA in addition to other peptides broken down by TCA itself.

The pH 4.6 and 6% TCA soluble extracts were correlated to assess their association or relationship. The two extracts were strongly correlated ($R^2 = 0.93$) indicating that the RP-HPLC method is useful for the detection of proteolysis in milk in both extracts.

Although results from this study confirmed that milk heated at 85°C for 15 s indicated higher proteolysis than raw milk, the TNBS method indicated higher proteolysis in raw milk than milk heated at 85°C (Chove et al., 2013). This could be explained as being due to the difference in the measurement principles of the methods. Le et al. (2006) explained that the HPLC method measures the absorbance at a given wavelength of separated peptides and amino acids, the response of which is based on mass and therefore the sensitivity for detection of larger peptides is greater than that for small peptides. The TNBS method measures the free amino groups, thus the higher the amino groups the greater the absorbance, irrespective of the amino acid composition (Beeby, 1980). The latter method is therefore more sensitive to small peptides than large peptides and proteins (Le et al., 2006).

Conclusion

The current study described the effect of high temperature processing of milk samples and its susceptibility to proteolysis during storage. Milk heated at higher temperatures (>85°C) had lower proteolysis than raw milk and samples heated at 85°C possibly due to destruction of the plasmin enzyme, which is a heat resistant enzyme. It was also shown that higher proteolysis in milk heated at 85°C for 15 s than raw milk was probably due to inactivation of plasmin and plasminogen inhibitors. Thus, it may be concluded that the higher the temperature employed for heating the milk, the less susceptible to proteolysis are the samples. This means that UHT milk processed using the temperature-time combinations employed in this study, is less susceptible to proteolysis than raw and milk heated at pasteurisation temperatures such as 85/15s. RP-HPLC method is a useful technique to detect proteolysis in milk heated at high temperatures.

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Physicochemical properties and consumer acceptability of soft unripened cheese made from camel milk using crude extract of ginger (*Zingiber officinale*) as coagulant

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This study was conducted to analyze the quality attributes of soft unripened cheese (SUC) made using ginger (*Zingiber officinale*) crude extract (GCE) by comparing it with cheese made using camel chymosin (CC). SUC made using GCE had higher acidity and ash but lower fat, total solids and protein as compared to cheese made using CC. The camel chymosin resulted in higher cheese yield as compared to cheese made using GCE. Although, lower than the values for cheese made using CC, sensory scores of cheese made using GCE is in line with literature values for camel milk cheese. The results indicated that GCE can be used to coagulate camel milk and thus help make cheese from camel milk.

Key words: Camel milk, cheese, ginger crude extract, physicochemical properties, sensory properties.

INTRODUCTION

Dromedary camels (*Camelus dromedarius*) produce more milk of high nutritional value that can be kept for a longer period of time as compared to other species in a hostile environment (Khan and Iqbal, 2001). Although camel milk has been consumed fresh in traditional pastoral systems for several centuries, processing of camel milk into more shelf stable value added milk products is not well developed and camel milk products are not common (Mehaia, 2006; Farah and Bachmann, 1987). Although coagulation of camel milk is reported to be difficult without addition of commercial milk clotting enzymes (FAO, 2001), possibilities of cheese-making from camel milk were reported by several authors (Khan et al., 2004; Mehaia, 2006; Ahmed and El Zubeir, 2011).

Recently, camel chymosin was developed using recombinant DNA technology by Danish scientists (Kappeler et al., 2006). However, it is not easily available for pastoralists or small camel milk processers; when available, it is not affordable. Generally, cheese making is reportedly curtailed by factors such as high cost and limited availability of calf rennet (Jacob et al., 2010; Hashim et al., 2011a). This calls for the need to search for alternative coagulants from easily available and cheap

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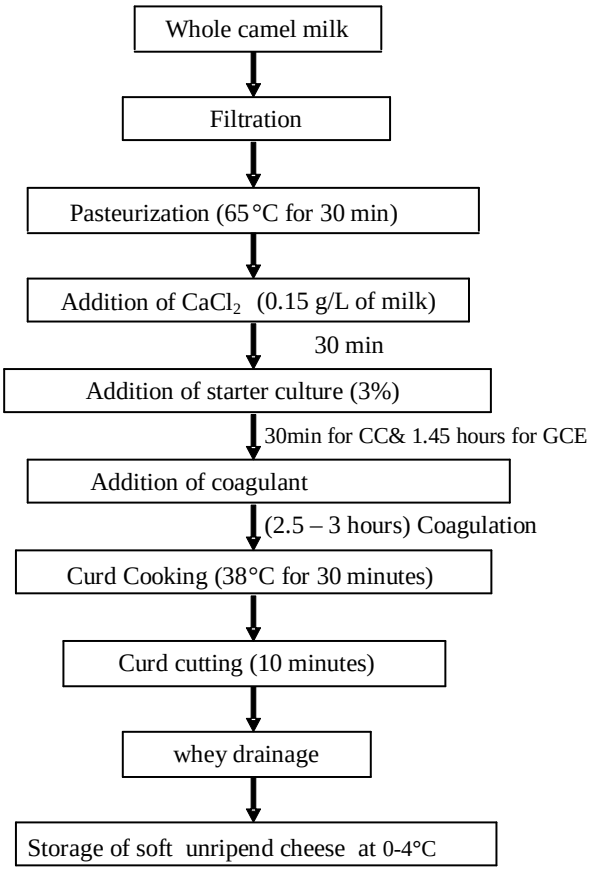

Figure 1. Flow diagram for the manufacture of soft unripened cheese from camel milk.

resources such as plant extracts to coagulate camel milk and make cheese out of it. Ginger extract was used to coagulate cow milk in which the milk clotting activity was caused due to the proteolytic activity of protease enzymes (Huang et al., 2011; Hashim et al., 2011a, b). Further, Hashim et al. (2011a) revealed that ginger extracted enzyme had high specificity for *α*-casein followed by *β*-casein and κ-casein and exhibited a similar affinity for κ-casein, α-casein and *β*-casein. Its higher specificity for κ-casein with increasing temperature was also reported (Huang et al., 2011). The milk clotting activity of ginger crude extract was reported to be higher than that of calf rennet and papain but lower than mucor rennet (Hashim et al., 2011a). This study was, therefore, conducted to assess the physicochemical properties and consumer acceptability of soft unripened cheese made by coagulating camel milk using ginger crude extract.

MATERIALS AND METHODS

Sample collection and physicochemical properties of camel milk

Camel milk samples were obtained from pastoralists living in the Erer valley in Eastern Ethiopia and delivered to Haramaya University Dairy Technology Laboratory by placing it in an ice box. The milk was collected by directly milking into sterile bottles. The extraction of ginger crude extract was performed according to the procedure described by Hashim et al. (2011a) with some modification as follows. Briefly, fresh ginger rhizomes were peeled, chopped, washed with de-ionized water and frozen at -23°C, then homogenized using a blender (Model 38BL40, Blender 8010E, Christiano Scientific Equipment, USA) with five parts of cold acetone (w/v) (-23°C) and kept at 4°C for about 15-20 min. The homogenate was filtered through cotton cloth and the precipitate was further washed with cold acetone followed by air drying. The air dried material was made into powder using a food grinding mill (Model M20, KIKA®WERKW, Germany). The powder was then homogenized in 20 mM phosphate buffer (pH 7.0) for 2.4 minutes (Hashim et al., 2011b) and the extract was filtered using a muslin cloth. The filtrate was centrifuged at 12,000 g for 20 min (Model 1020, D Centrifuge, Centrurion Scientific LTD) and the supernatant was considered as crude extract. Liquid camel Chymosin (Chy-Max® M, Christian Hansen, Denmark) with an activity of 1000 IMCU/ml at a concentration of 0.1 ml/L was also used for manufacturing of soft unripened cheese for comparison purpose. A yoghurt culture (*Streptococcus thermophilus*) (Micro-Milk, Italy) was used for acidification of milk. The physicochemical properties (pH, titratable acidity, total solids, ash, protein and fat) of raw camel milk were analyzed using standard procedures (AOAC, 1995).

Manufacturing of soft unripened cheese

Soft unripened cheese was manufactured from camel milk according to the method described by Mehaia (2006). Three different batches of cheese were made. At the end of each manufacturing process, the cheese samples were collected in sterile containers and kept in refrigerator at 4°C for further analysis. Ten liters of camel milk brought to the laboratory was divided into two lots of five liters each (ginger crude extract was used to coagulate one of the lots, added at a pH value of 5.2 and camel chymosin was used to coagulate the second lot and added at a pH value of 6.2, which served as a control). Both lots were inoculated simultaneously with the yoghurt starter culture, *S. thermophilus* (3%). Calcium chloride (0.15 g/L of milk) was added into the milk 30 min prior to the addition of the coagulants as recommended by FAO (2001). The other subsequent steps were maintained the same during cheese-making for both treatments (Figure 1).

The physicochemical properties (pH, titratable acidity, total solids, ash, protein and fat) of soft unripened cheese (SUC) were analyzed following standard procedures (AOAC, 1995). pH and titratable acidity were measured in the same day the cheese was manufactured; however, the other parameters were analyzed within 48 h of manufacture of the cheese. The cheese yield was calculated as suggested by Mehaia (2006) and was expressed as kg/100 kg of milk. Cheese yield = weight of cheese/weight of the milk sample.

Sensory evaluation

Thirty voluntary panelists were selected based on the criteria suggested by Hashim (2002): age between 18 and 34 years, and usual consumers of camel milk or fermented camel milk and cheese from milk of other species to rate the acceptability of the cheese based on its color, appearance, aroma, taste, texture and overall acceptability using a 7-point hedonic scale (1 = dislike extremely, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately and 7 = like extremely). Each batch of cheese was coded with three digit numbers and provided to panelists after 24 h of manufacturing. Fifteen grams of soft

Table 1. Physicochemical properties of camel milk samples used for making soft unripened cheese.

Variables	Values
pH	6.64 ± 0.02
Titratable acidity (% lactic acid)	0.15 ± 0.01
Total solids (%)	11.6 ± 0.27
Ash (%)	0.94 ± 0.03
Fat (g/100ml)	3.0 ± 0.10
Protein (g/100 ml)	2.9 ± 0.13

Table 2. Physicochemical properties of soft unripened cheese made from camel milk using crude extracts of ginger and chymosin as coagulant.

Variables	Cheese made using ginger crude extract	Cheese made using chymosin
pH	4.87 ± 0.04^{b}	5.27± 0.02^{a}
Titratable acidity (% lactic acid)	0.81 ± 0.06^{a}	0.57 ± 0.06^{b}
Total solids (%)	35.40 ± 0.03^{b}	39.90 ± 1.76^{a}
Ash (%)	2.10 ± 0.04^{a}	1.98 ± 0.00^{b}
Fat (g/100 g)	12.90 ± 0.14	13.40 ± 0.59
Protein(g/100 g)	16.40 ± 0.54^{b}	19.70 ± 0.89^{a}
Actual yield (kg/100 kg milk)	8.70 ± 1.22^{b}	11.4 ± 0.36^{a}

Means with the same superscript letters within a row are not significantly different (P > 0.05); Values in the table are mean ± SD of three replications.

unripened cheese was allotted to each judge. Sensory evaluation was conducted in a well-ventilated and illuminated room.

Data analysis

The physicochemical and sensory score data were analyzed using the ANOVA technique of completely randomized design. Means were separated using least significant difference method.

RESULTS AND DISCUSSION

Physicochemical properties of soft unripened cheese made from camel milk

The whole camel milk used for cheese making had pH 6.64 ± 0.02, titratable acidity 0.15± 0.01, percent total solids 11.6 ± 0.27, percent ash 0.94 ±0.03, fat 3.0 ± 0.10 g/100ml and protein 2.9 ± 0.13 g/L (Table 1). Significant difference (P<0.05) was observed in physicochemical properties of cheese samples made from camel milk using ginger crude extract (GCE) and camel chymosin (CC) (Table 2).

In the present study, lower pH values were observed for SUC made from camel milk as compared to values reported in literature. As indicated by El-Aziz et al. (2012), pickling of cheese from buffalo milk using ginger extract resulted in a reduced pH (from pH of 5.86 to 4.94). Inayat et al. (2003) reported a comparable pH value (5.23) for SUC made from camel milk as that made using CC in the present study. The higher titratable acidity recorded in the present study for SUC made using GCE, might be due to the type of coagulant added, temperature used and the time that elapsed in the cheese-making process that has an effect on the acidification process.

Higher total solids were observed for SUC made using CC as compared to SUC made using GCE (Table 2). Khan et al. (2004) and Mehaia (1993) indicated higher values of total solids (44.4 and 45.5% respectively) for cheese made from whole camel milk as compared to the results of the present study. Lower ash content was observed for SUC made using CC when compared with SUC made using GCE (Table 2). Lower values of ash content were reported for soft white cheese manufactured from camel milk by Khan et al. (2004) (1.53%), Ahmed and El Zubeir (2011) (1.53%) and Mehaia (1993) (1.85%) when compared with that observed in the current study.

Several factors can be responsible for these differences that, among others, include the nature of ingredients used in the cheese-making process and the ash content of the raw material used. Mehaia (2006), for instance, indicated that addition of salt during cheese manufacturing resulted in higher ash content of Domiati cheese.

Higher protein and cheese yield values were observed for SUC made using CC as compared to SUC made using GCE (Table 2). Inayat et al. (2003) reported a

Table 3. Mean sensory scores of camel milk cheese made using crude extract of ginger and camel chymosin as coagulants (n= 30).

Sensory attributes	Cheese made using ginger crude extract	Cheese made using camel chymosin
Color	5.20 ± 0.96[b]	6.50± 0.68[a]
Appearance	4.70± 1.26[b]	6.17± 0.83[a]
Aroma	5.93± 0.79[a]	5.20 ±1.27[b]
Taste	5.00± 1.34[b]	5.70± 1.09[a]
Texture	4.83± 1.12[b]	5.67 ± 1.47[a]
Overall acceptability	5.23 ± 0.94[b]	6.47± 0.68[a]

Means with the same superscript letters within a row are not significantly different (P > 0.05); n = total number of sensory panelists.

comparable value of protein (19.64%) for soft unripened cheese made from camel milk as to the cheese made using chymosin in the present study. Hashim et al. (2011a) and Huang et al. (2011) indicated high proteolytic activity of ginger extracts on milk casein. Therefore, the variations in protein contents between the cheese types in the present study could have occurred due to the hydrolytic activity of the coagulant used for the cheese-making. Bansal et al. (2009), for example, reported that Cheddar type cheese made from camel chymosin resulted in low proteolysis as compared to cheese made with calf chymosin. El-Aziz et al. (2012) also reported that cheese proteolysis could be enhanced by the use of ginger crude extract as a coagulant for cheese making from buffalo milk.

Weak firmness of curd was observed for milk treated with crude extracts of ginger and resulted in smaller curd particles, which were not well coagulated; thus escaped out with the whey through the pores of the muslin cloth. This might be the reason for low cheese yield observed for milk treated with GCE. According to the report of Kamoun and Bergaouin (1989), cheese yield of 12 kg was obtained from 100 kg of camel milk. Cheese yield of 13.22 and 14.9% were also reported by Khan et al. (2004) and Mehaia (2006), respectively for fresh white cheese made from camel milk.

Sensory evaluation of soft unripened cheese made from camel milk

Significant differences (P<0.05) were observed for all sensory attributes between cheeses made using GCE and CC (Table 3). Cheese made using GCE had lower score for color than that made using CC (Table 3). According to Delahunty and Drake (2004), appearance of cheese is a function of the interaction between cheese color and texture, and coagulant type used.

On the other hand, in the present study, cheese made using GCE had higher score for aroma as compared to cheese made using CC (Table 3). This variation in cheese aroma might be attributed to the inherent property of the crude extract of ginger rhizome (aromatic plant used as a spice) that provides a pleasant aroma and enhances the flavor of the cheese (El-Aziz et al., 2012).

In the present study, cheese made using CC had higher scores for texture and overall acceptability than cheese made using GCE (Table 3). Bansal et al. (2009) suggested the less primary proteolysis by camel chymosin to be a possible reason for acceptable texture of Cheddar cheese made using camel chymosin. Proteolytic activity of ginger contributed to textural softening during ripening of Iranian ultra-filtrate white cheese (Fathollahi et al., 2010). El-Aziz et al. (2012) indicated that fortification of buffalo milk with ginger extract caused an increase in cohesiveness and a decrease in firmness of the curd, which resulted in more softness and smoothness during storage of cheese but high score for overall acceptability of cheese.

Although, the sensory score values for most of the sensory attributes were lower for cheese made using GCE than that made using CC, the values for sensory attributes observed in cheese made using GCE are higher than sensory scores reported by earlier researchers for camel milk cheese. Benkerroum et al. (2011) reported mean sensory scores of 4.30, 4.15, 4.25 and 4.50 for color, texture, flavor and acceptability for camel milk cheese obtained using chymosin (Chy.Max^{TM}) as a clotting agent.

Conclusions

The results of the current study revealed the possibility of making SUC from camel milk by coagulating it using locally available ginger crude extract. Although, the values for most of the quality parameters of SUC made using GCE were lower than the corresponding values for cheese made using the commercial camel chymosin, the observed result is promising and shows possibilities of improving the quality of such cheese in the future. Production of cheese from camel milk using easily available and cheap coagulants such as ginger crude extract is of paramount importance in adding value to camel milk. Further studies are needed to confirm the result and improve the outcomes using pure enzyme from ginger.

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Effect of boiling and frying on nutritional value and *in vitro* digestibility of rabbit meat

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Effect of boiling and frying on nutritional value and *in vitro* digestibility of rabbit meat were investigated. It was observed that boiling for 15 min increased the digestibility and nutritional value of rabbit meat, while boiling for 5 and 40 min led to their loss. Although, frying for 2 and 4 min produced an acceptable digestibility and nutritional value, the values were lower than those of the boiled rabbit meats. These results were supported by the analysis of amino acid and SDS-PAGE. Boiling or frying for longer time led to decrease in whiteness and protein content of rabbit meat. Micrographs showed that the meat myofibrils became clearer with the increase in boiling time, but a fuzzier surface appeared with the increase in frying time. From these results, it was concluded that a proper boiling or frying time is necessary to acquire a nutritious and high quality rabbit meat.

Key words: Rabbit meat, boiling, frying, amino acid, protein, essential amino acid index (EAAI).

INTRODUCTION

Rabbit meat has a high protein and low sodium contents (Combes, 2004). Its cholesterol and fat contents are much lower than chicken, turkey, beef and pork (Combes, 2004; Cavani et al., 2010). The unsaturated fatty acids content of rabbit meat is 63% (w/w) of its total fatty acids. The United States Department of Agriculture (USDA) has stated that the rabbit meat is the most nutritious meat available to man (Cavani et al., 2010). The rabbit meat was highly accepted for its appearance and color, juiciness and taste when boiled and fried as comparing to the Giant African Land Snail (*Archachatina marginata*) meat (Malik et al., 2011). All these features make rabbit meat a very healthy, nutritious and popular food suitable for general consumption and as a special diet including heart disease patients diet, low sodium diet, weight reduction diet, diets for the aged, etc (Dalle, 2002; Cavani and Petracci, 2004).

Cooking is necessary to prepare palatable and microbially safe foods including rabbit meat. Suitable cooking minimizes the nutrient loss and also improves the digestibility of food (Kimura and Itokawa, 1990). However, improper cooking will terribly harm the nutrients present in the food, and sometimes even produce toxic and hazardous substances. Investigations have demonstrated that there were positive associations between red processed meat and proximal colon cancer, pan-fried red meat and colorectal cancer (Miller et al., 2013), consumption of fried and/or well-done red meat was associated with increased risk of lung cancer in women (Sinha et al., 1998). Carcinogenic compounds formed by high-temperature cooking techniques like heterocyclic amines and polycyclic aromatic hydrocarbons were reported to contribute to the risk of developing colorectal tumors (Sinha et al., 1999; Wong et al., 2012). Meat cooked with high temperature techniques produced mutagenic compounds such as heterocyclic amines, which are strongly associated with the risk of colorectal adenomas (Sinha et al., 1999; Wong et al., 2012). The degree of browning that occurred during deep-frying of meat was related to the pathogenesis of breast cancer (Dai et al., 2002; Lyn-Cook et al.,

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2011) and a high intake of processed meat (pork chops, sausages and hotdogs) was found to increase the risk of lung cancer in men (Tasevska et al., 2009). Fried meat was associated with a higher risk of cancer of oral cavity, pharynx and esophagus, roasting/grilling meat increased risk of prostate cancer (Di Maso et al., 2013).

Due to the health concerns caused by improper cooking of meat, cooking method has been paid special attention and many studies have been done to investigate the effects of heating on meat quality and its nutrients. It was found that the conventional and microwave heating of ground beef samples increased the nonheme iron concentrations (Schricker and Miller, 1983) and the fried ground beef patties had the highest flavor intensity (Berry and Leddy, 1984). The amount of heterocyclic amines increased with frying or boiling time increase during the production of braised chicken (Yao et al., 2013). Cooking had no significant effect on riboflavin content of dark meat, while frying significantly decreased the riboflavin content of light meat (Al-Khalifa and Dawood, 1993). The combination of roasting and microwave heating of hamburgers increased the lipid oxidation (Rodriguez-Estrada et al., 1997). Heat cooking considerably affected the proximate composition and mineral contents and baking and grilling were reported as the best cooking methods for rainbow trout (Gokoglu et al., 2004).

Effect of cooking on rabbit meat quality had been investigated in the past. Some of the investigations include the effect of electric oven cooking on oxidative stability of meat of rabbit fed with fodder containing α-tocopheryl acetate (Monahan et al., 1990). The effect of boiling (in a vacuum sealed polyethylene bag at 100±1°C for 8 min), frying (in sunflower oil at 175±5°C for 3 min), roasting (in an electric oven preheated to 200±10°C for 15 min) on pH, shear force, lightness, color, and water holding capacity of rabbit meat (Bosco et al., 2001), was also investigated. The effect of cooking (immersed in a constant temperature water-bath) temperature (50-90 °C) and time (10-120 min) on tenderness of rabbit muscle had been investigated (Combes et al., 2004). Recently, the effect of cooking methods and the related processes on nutrition and quality of chicken (Das et al., 2013), silver carp (Naseri et al., 2013), beef muscles, rainbow trout fillets (Choubert and Baccaunaud, 2010), and beef roast (Modzelewska-Kapituła et al., 2012; Walsh et al., 2010), were investigated. However, to our knowledge, the effect of cooking methods on the protein content and quality of protein present in the cooked rabbit meat have not been investigated or reported previously. Better knowledge of effect of cooking methods on the nutritional value of protein of rabbit meat will contribute to the development of nutrient rich rabbit meat products.

Heat cooking is being used as a general term for various processes including boiling, frying, roasting, baking, etc. Boiling and frying are most frequently used for cooking of meat (Fillion and Henry, 1998; Chen, 2006). However, the effect of doneness or cooking levels on protein qualities of rabbit meat was rarely investigated. This research is focused on investigating the effect of two commonly used heat cooking methods (boiling and frying) and different doneness levels on nutritional value of protein and *in vitro* digestibility of rabbit meat.

MATERIALS AND METHODS

Reagents

Amino acid standards used in this study were obtained from Sigma-Aldarich Corporation (St. Louis, MO). HPLC grade acetonitrile and methanol were used in this study. The trichloroacetic acid (TCA) was purchased from Jiangsu Yonghua Fine Chemical Co., Ltd (Shanghai, China). Sodium dodecyl sulfate (SDS), urea, Tris (hydroxymethyl) aminomethane (Tris), β-mercaptoethanol (β-ME) and other electrophoresis reagents were purchased from Shanghai Bio Life Science and Technology Co., Ltd (Shanghai, China). All chemicals used in this study were of analytical grade.

Meat samples

Totally, 140 numbers of 10 week old Sichuan Rex Rabbits, having a body weight of 2.35±0.1 kg were used in the experiments. The rabbits were slaughtered by the butchers of Hage Rabbit Industry Co. Ltd, in Sichuan Yilong County and brought to our laboratory within 2 h. The rabbit meat samples for cooking experiments (boiling and frying) were taken from hind legs. The meat was chopped into dice having uniform size of 20 ± 3 mm in both length and width, and 10 ± 3 mm in height. A total weight of 300 g hind leg meat was used for each treatment. Six treatments (boiling for 5, 15 and 40 min; frying for 2, 4 and 6 min) were performed for 9 times each.

Cooking methods

In order to investigate effect of heat cooking methods on nutritional value and *in vitro* digestion of rabbit meat, the diced meat was boiled and also fried using the procedure shown below. The boiling of the rabbit meat was done with six times volume of hot water in a temperature-controlled media MK2102 electromagnetic furnace (Media Group, China) at temperature of 95±3°C for a predetermined time of 5 (equals to medium), 15 (equals to well done) and 40 min (equals to very well done). The rabbit meat was fried with 1/3 times soybean oil in a temperature controlled Media MK2102 electromagnetic furnace (Media Group, China) set at 175±3°C for 2 (equals to medium), 3 (equals to well done) and 6 min (equals to very well done).

Determination of moisture and protein

Moisture contents of samples were analyzed according to the Association of Official Analytical Chemists method (AOAC 950.46, 1990). The crude proteins (N = 6.25) and dry matter contents were analyzed according to Association of Official Analytical Chemists (AOAC, 2006). All the tests were conducted in triplicate.

Color analysis

CIELAB color (L*, a* and b*) of cooked muscles were tested using a portable chroma meter CR-400 (Minolta Cameras, Osaka, Japan). The instrument was calibrated using the white

plate (Calibration Plate CR-A43, Minolta Cameras) at the beginning of each session. The chroma meter has a measuring area of 8 mm in diameter and 8 mm in thickness and illuminates the sample area with the diffused illumination from a pulsed xenon arc lamp. After choosing the Commission International illuminant (CIE) lighting conditions, the calibration channel selection was set at auto select mode. Reflectance measurements were obtained at a viewing angle of 0° and the spectral component was included. Color is defined by the three CIE coordinates, L* (lightness), a* ((+) redness/ (-) greenness) and b* ((+) yellowness/ (-) blueness). The whiteness (W) is calculated from the L*, a*, b* values using the equation (Lanier et al., 1991).

$$W = 100-((100-L^*)^2+a^{*2}+b^{*2})^{0.5}$$

The cooked samples were dried, milled and filled in a 40 × 25 mm weighing bottle. The powder in the weighing bottle was placed smoothly in the plates and the color was determined using the chroma meter. Six repeated determinations were done for each sample.

Analysis of the micro-structure of meat in optical microscopy

The surface micro-structure of cooked and uncooked rabbit meat was observed with a XSP-15 reading optical microscope (Jiangsu Optical Instrument Factory, Nanjing). In order to maintain the natural state, the meat samples were not treated with any reagents. The meat sample was fixed with the stage clips and was observed under diffused reflection light with a 10 times magnifying eyepiece lens and 16 times objective lens. The optical micro surface structure was obtained by an IXUS 60 6.0 MP Digital Camera.

Sequential *in vitro* protein digestion procedure

The *in vitro* digestibility of the proteins was evaluated by the method used by Fu et al. (2002), with some modification. Pepsin (Sigma, P7000, 1:10,000, 600-1000 units/mg) and trypsin (Genview, DH355-1, 1:250) were used for the *in vitro* digestion study. Five grams of meat sample from each treatment (boiled or fried meat were converted as the weight of uncooked) was chopped into pieces and dipped into 250 mL solution of hydrochloric acid (HCl) of pH 1.5. The dipped samples were pre-incubated in a water bath at 37°C for 3-5 min. Then, an amount of pepsin (20 mg of pepsin per mL of 0.1 M KH_2PO_4, pH 2, buffer) was added, and the ratio of enzyme to protein substrate was maintained at 1:100 (w/w). The pepsin and protein solutions were mixed well and incubated at 37°C. After the predetermined incubation periods: 0, 60, 120, 180, and 300 min, aliquots of the mixtures were taken, and the pH was adjusted to pH 7.0 by adding 1.0 mol/L NaOH to stop the enzymatic reactions. Additionally, the final pepsin-digested hydrolysates (pH 7.0) were further digested by the addition of trypsin (20 mg of trypsin per mL of 0.1 M Tris-HCl buffer, pH 7.0) at 37°C for 60, 120, 180, and 300 min. The amount of enzyme to initial protein substrate ratio was maintained at 1:20 (w/w).

Nitrogen release during digestion

The nitrogen release (%, w/w) during digestion was determined by the TCA-NSI method used by Iwami et al. (1986), with some modifications. 10 mL of the digested mixtures were mixed with 10 mL of 10% (w/v) TCA (and the final concentration of TCA was 5%, w/v). The mixtures were then centrifuged (20°C, 8000 g, 30 min) to obtain the precipitates. After washing with 10 mL of TCA (10%, w/v), the precipitates were obtained again by centrifugation at the same condition. The N content of the samples was determined by Kjeldahl method (N×6.25). The nitrogen release (%, w/w) during the digestion was calculated as:

$$\text{Nitrogen release} = (N_0-N_t)\times 100/N_{total}$$

Where, t is the digestion time (min), N_t (mg) is the TCA-insoluble N after digestion for t (min), N_0 (mg) is the TCA-insoluble N in the protein sample and N_{total} (mg) is the total N of protein sample. Triplicate determinations were done for each sample.

Amino acid analysis

The amino acid composition of the rabbit meat samples were determined using the method used by Bidlingmeyer et al. (1984). The samples were hydrolyzed with a 6 M HCl solution in a vacuum-sealed tube for 24 h at 110°C. The samples were subsequently centrifuged (1500 rpm for 5 min) and dried under vacuum for 1.5-2 h. The pH was adjusted by adding 20 µL of an ethanol : water : triethylamine (2:2:1) solution and the samples were dried for 1.5-2 h. The resulting sample was derivatised by adding 20 µL of ethanol : water : triethylamine : phenylisothiocyanate (7:1:1:1) derivatising solution which was then allowed to react at room temperature for 10 min prior to drying under vacuum (minimum of 3 h). The samples were re-suspended in 200 µL of Picotag sample diluent (Waters, Millford, MA, USA) and 8 µL sub-sample was injected for separation by HPLC under gradient conditions. Buffer A was a sodium acetate buffer (pH 6.4) containing 5000 ppm ethylenediaminetetraacetic acid (EDTA), 1:2000 triethylamine and 6% acetonitrile and buffer B consisted of 60% acetonitrile with 5000 ppm EDTA. A waters high performance liquid chromatography system (1525 HPLC with a binary gradient delivery, 717 auto-sampler and Injector, 1500 column heater, 2487 dual wavelength UV detector) and a Breeze data workstation (Waters, Millford, MA, USA) were used for the determination of amino acid composition. Triplicate determinations were done for each sample.

Nutrition analysis of amino acid

Biological values of cooked rabbit meat was analyzed by 5 parameters; they are total amino acid (TAA) content, essential amino acid ratio (EAAR), protein chemical score (PCS), essential amino acid index (EAAI) (FAO., 1985) and protein efficiency ratio (PER) (Alsmeyer et al., 1974). The specific calculation formula of those parameters as follow:

$$TAA = \sum_{i=1}^{n} a_i$$

$$EAAR = \sum_{j=1}^{m} b_j \Big/ \sum_{i=1}^{n} a_i$$

$$PCS = Min\{(aa/AA)_1,\cdots,(aa/AA)_k\}$$

$$EAAI = \sqrt[k]{(aa/AA)_1\times\cdots\times(aa/AA)_k}$$

$$PER = -0.468+0.454\times leucine-0.105\times tyrosine$$

where a - amino acid in meat sample; b - essential amino acid in meat sample; n - the number of amino acid; m - the number of essential amino acid; AA - the content of amino acid in FAO (1985)

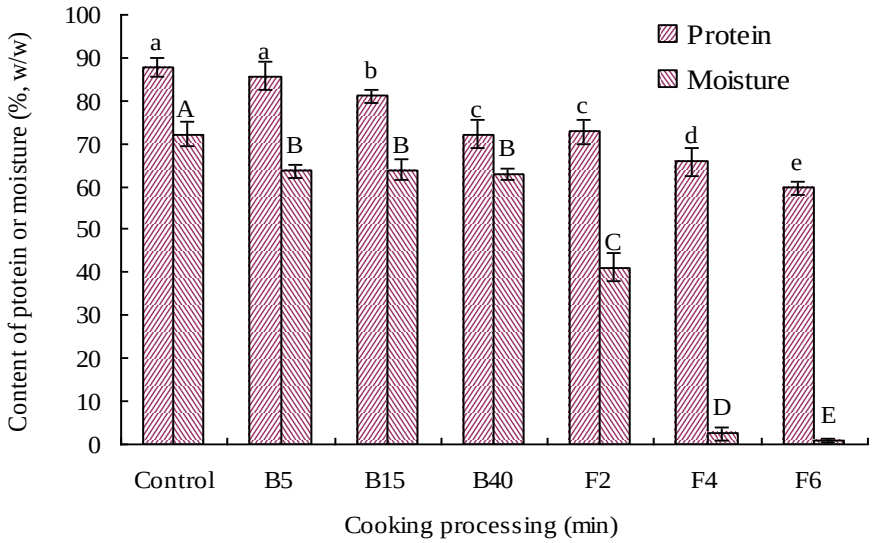

Figure 1. Effect of heat cooking on content of protein and water of rabbit meat. Protein content was calculated on dry basis; Means (n=3) without a common letter differ significantly (P<0.05); "Control" is uncooked rabbit meat, B5, B15 and B40 represents meats boiled for 5, 15, and 40 min, respectively; and F2, B4 and B6 represents meats fried for 2, 3 and 6 min, respectively.

suggested pattern of protein requirement; aa - the content of amino acid as compared to FAO (1985) suggested pattern of protein requirement and k- the number of amino acid type (FAO, 1985, Alsmeyer et al., 1974).

SDS–polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE analysis was performed according to the method of Laemmli (1970). Two grams of cooked meat sample was added to 18 mL of SDS solution (5 g/100 mL). The mixture was then homogenized using a FJ-200 homogenizer (Shanghai Specimen and Model Factory, Shanghai, China) at a speed of 10,000 rpm for 1 min. The homogenate was incubated at 85°C in a temperature-controlled water bath for 1 h to dissolve total proteins. The sample was centrifuged at 12,000 g for 6 min. Solubilized samples were mixed at 1:1 (v/v) ratio with the sample buffer (0.5 mole/L Tris-HCl, pH 6.8, containing SDS [4 g/100 mL], glycerol [20 g/100 mL] and β-ME [10 g/100 mL]), and the mixture was boiled for 3 min. The samples (20 mg protein) were loaded into the polyacrylamide gel made with 10% running gel and 4% stacking gel, and were subjected to electrophoresis at a constant current of 15 mA. After separation, the proteins were stained with Coomassie Brilliant Blue R-250 (0.02 g/100 mL) in methanol (50 mL/100 mL) and acetic acid (7.5 mL/100 mL), and destained with methanol (50 ml/100 ml) and acetic acid (7.5 mL/100 mL), followed by methanol (5 mL/100 mL) and acetic acid (7.5 mL/100 mL). The electrophoresis gel was scanned with a Bio-Rad imaging scanning densitometer (Versa Dco 3000, Bio-Rad, Milan, Italy).

Analysis of electrophoresis gel

The scanned electrophoresis gel image (300 dpi) was subjected to the optical density (O.D) analysis. Optical density is the measure of the transmission of an optical medium for a given light. Higher O.D indicates lower transmittance and vice versa. The O.D of the electrophoresis gel image was determined by a Gel-Pro Analyzer 4.0 (Media Cybernetics, L. P., USA). The O.D is calculated using the equation given below:

$$OD(x, y) = \frac{-\log(INTENSITY(x, y) - BLACK)}{INCIDENT - BLACK}$$

Where: INTENSITY (x, y) is the intensity at pixel (x,y); BLACK is the intensity generated when no light goes through the material; INCIDENT is the intensity of the incident light.

Statistical analysis

The results were analyzed by ANOVA at a significance level of 5% (H0: P<0.05). The comparison of means was analyzed by Fisher's LSD tests using the SAS statistical package (Statistical Analysis Systems Institute (SAS), 2000).

RESULTS AND DISCUSSION

Effect of heat cooking on protein and moisture contents of rabbit meat

The protein and moisture contents of the raw (control) and the cooked rabbit meats are shown in Figure 1. Both boiling and frying reduced the moisture content of rabbit meat significantly (P<0.05) as compared to the control, but the rate of moisture reduction is significantly (P<0.05) higher for frying than boiling. Boiling of rabbit meat for 5 (B5), 15 (B15) and 40 min (B40) caused a moisture reduction of 12.03, 11.66 and 12.98%, respectively. The frying of rabbit meat for 2 (F2), 4 (F4) and 6 min (F6) resulted in the moisture reduction of 49.92, 96.56 and

Table 1. Effect of cooking on color of rabbit meat.

	Control	B5	B15	B40	F2	F4	F6
L*	64.82±0.07a	64.54±0.39a	63.31±1.46b	62.20±0.44c	47.38±0.25d	36.26±0.83e	21.50±0.14f
a*	-1.95±0.12d	-2.85±0.11e	-1.64±0.58d	-2.82±0.48e	4.40±0.15b	12.52±0.14a	3.49±0.19c
b*	25.48±0.34c	24.32±0.05 d	24.26±0.44d	26.04±0.52c	37.54±0.79a	28.18±0.35b	2.21±0.18e
W	56.52±0.26 a	56.90±0.33a	55.97±1.01a	54.01±0.30b	35.21±0.59c	29.19±0.73d	21.40±0.14e

Each data is a mean of three replicate; Means (n=3) without a common letter differ significantly (P<0.05); "Control" is uncooked rabbit meat, B5, B15 and B40 represents meats boiled for 5, 15 and 40 min, respectively; F2, F4 and F6 represents meat fried for 2, 4 and 6 min, respectively; L* (lightness), a* ((+)redness/(-)greenness) and b* ((+)yellowness/(-)blueness), W (whiteness).

98.88%, respectively. The above results showed that the boiling has less influence on moisture content of rabbit meat as compared to frying which led to severe moisture loss to the extent of 98.88% depending on the frying time. Similar results were reported for boiling and frying of rainbow trout meat by Gokoglu et al. (2004), who found that frying and boiling had considerable effect on the proximate composition and mineral contents of the cooked fish comparing with the raw meat.

All the heat cooking treatments performed in this study resulted in significant loss of protein content of rabbit meat as compared to the control except the boiling for 5 min (B5). The loss of protein content of rabbit meat after boiling for 5 (B5), 15 (B15) and 40 min (B40) were 2.51, 7.75 and 17.78%, respectively. These results are in agreement with the results obtained by Bosco et al. (2001) for the loss of crude protein of rabbit meat during boiling. The protein loss during frying treatments of 2 (F2), 4 (F4) and 6 min (F6) were 17.20, 25.17 and 32.06%, respectively. The loss of protein content increased with an increase in both boiling and frying times and the loss caused by frying is greater than that of boiling. Frying resulted in rabbit meat with a low protein content similar to the results obtained by Oz et al. (2007). New chemicals, such as heterocyclic amines and polycyclic aromatic hydrocarbons, were formed during the frying might be the reason of the low protein content of fried rabbit meat. Those chemicals have been confirmed to contribute to the risk of developing colorectal tumors (Sinha et al., 1999; Wong et al., 2012), cancer of oral cavity and pharynx and esophagus (Di Maso et al., 2013).

Water molecules are highly polar and attracted to the muscle proteins by ionizable basic and acidic groups such as arginine, histidine, lysine, glutamic acid, etc (Wierbicki and Deatherage, 1958). The meat protein was denatured by heating and a part of its ionized basic or acid groups were broken. This led to the release of the water molecules in meat, which resulted in low moisture content of cooked meats than that of the control. The moisture loss of fried meats was more than those of boiled ones. The possible reason for this might be that the high temperature frying (175±3°C) caused more and rapid denaturation of meat protein when compared with low temperature boiling, resulting in more loss of water molecules (Skog et al., 1995). Oil absorption during frying might be another reason for low moisture content of fried meats than that of the boiled meats.

Prolonged boiling caused hydrolyzes of part of connective tissue and other protein in meat (Mutilangi et al., 1996) and leaking out of sarcoplasmic proteins from the muscle fibers channel (Murphy and Marks, 2000). Hydrolysis of proteins and leaking out of sarcoplasmic proteins might be the reason for the higher loss of protein content from the meat boiled for 15, 40 min than the meat boiled for 5 min and the control. The increased loss of protein content with an increase in frying time was related to the loss of nitrogen in the form of volatile nitrogenous compounds during frying (Yu et al., 1993).

Effect of heat cooking on color of rabbit meat

The CIELAB color (L*, a*, b*) values obtained from the raw and cooked rabbit meat samples are shown in Table 1. The L* value of rabbit meat boiled for 5 min (B5) was similar to that of control, whereas the L* values of meat boiled for 15 (B15) and 40 min (B40) and fried for 2 (F2), 4 (F4) and 6 min (F6) were significantly (P<0.05) lower than that of control. The L* value decreased in the order of B15>B40>F2>F4>F6. The L* values showed that the lightness of the rabbit meat was less affected by boiling than the frying.

The a* values of meat obtained from frying treatments F2, F4 and F6 were significantly (P<0.05) higher than that of control, and the boiling treatments B5, B15 and B40 (Table 1). It was also found that a* of control, B5, B15 and B40 were negative, but the a* of F2, F4 and F6 were positive. The positive a* values indicated that the frying made the cooked meat to become red and boiling treatments maintained the greenness (-a*) of meat similar to that of control. There was no significant (P>0.05) difference observed in b* values for the boiling treatments B5, B15 and B40 as compared to control whereas the b* values of meat samples from frying treatments, F2, F4, F6 were found to be significantly (P<0.05) different from the control. The highest b* value was obtained for F2 and b* decreased with increase in frying time. The b* values

indicated that the frying had a greater influence on b* of meat than that of the boiling. No significant (P>0.05) difference in the whiteness values was observed for meat samples of control and treatments B5 and B15, but the whiteness values of meat samples of B40, F2, F4 and F6 were found to be significantly (P<0.05) different than the control. The whiteness values are found to be in the ascending order of B40>F2>F4>F6. These results showed that either boiling for longer time or frying for even shorter time (2 min) caused the meat to become darker and the frying severely affected the whiteness of cooked meat. These results are consistent with the findings of Lien et al. (2002) for heat processing of pork patties.

Higher moisture content in the meat increased the lightness of meat gels (Park, 2006). The boiled meat samples had a higher moisture contents than the fried meats, which might be the reason for the higher L* values of boiled meats than those of the fried ones. Previous findings showed that the higher moisture content decreased yellowness (b*) (Park, 2006) and higher cooking temperature decreased the b* value of cooked meat (Brewer and Novakofski, 1999). These findings did not help much to explain the changes in b* values for the boiled or fried meat samples. The low a* values of meat samples of low temperature treatments can be corroborated to the prior findings that red color in cooked meat diminished due to a masking of the myoglobin by aggregation/co-precipitation of other myofibrillar and sarcoplasmic proteins at lower temperatures (Brewer and Novakofski, 1999). The darker color of fried meat was also observed by Sosa-Morales et al. (2006), who reported that frying resulted in a darker crust color of pork meat. Higher redness values in fried products could also be attributed to concentration of meat pigments as moisture content was decreased and the Maillard reaction happened during meat frying, which resulted in the formation of heterocyclic amines (Wong et al., 2012) and have been confirmed to contribute to the risk of developing colorectal tumors (Sinha et al., 1999), deep-frying of meat resulted in a browning color (Dai et al., 2002; Lyn-Cook et al., 2011). All these might be a possible reason why the a* of the fried meat samples had positive a* values and also higher than those of the boiled meats.

The equation used for calculating the whiteness values from the observed L*, a*, and b* values illustrated that L* influences the whiteness more than a* and b*. Therefore, the whiteness of meat samples changed similarly to the change in the lightness value.

Effect of heat cooking on surface feature of rabbit meat

In order to differentiate surface feature of cooked meat samples, the micro structure of raw rabbit meat (control) and the cooked rabbit meats were determined (Figure 2). When compared with the control, the myofilament of the boiled meats (B5, B15 and B40) were clearly seen with the increase in boiling time, but the myofilament of the fried meats (F2, F4, F6) became fuzzier with the increase in frying time, and a black coagulation on the surface was observed, especially on the meat fried for 6 min.

Boiling and frying caused moisture loss of meat samples (Figure 1). Water molecules were attracted to the muscle proteins (Wierbicki and Deatherage, 1958) and heating led to denaturation of meat protein as discussed earlier. The water molecules bounded at myofilament were transferred from myofibrils to the extracellular space (Bertram et al., 2002). This transformation of water within the meat myofibrils and the denaturation of meat protein caused the shrinkage of meat within the tissue matrix, and resulted in the myofibrils standing out (Murphy and Marks, 2000). Therefore, the myofibrils of the boiled meats were seen clearly with increase in boiling times. The surface of the fried meat samples looked fuzzier with the increase in frying time. The possible reason for this could be that the high temperature during frying caused severe denaturation of the myofibrillar protein and some of the frying oil is absorbed in fried meat replacing water (Fillion and Henry, 1998). Maillard reaction happened during frying and formation of dark color heterocyclic amines (Wong et al., 2012) is another possible reason causing the natural surface of the rabbit meat to look tougher and fuzzier.

Effect of heat cooking on *in vitro* digestibility of rabbit meat

The *in vitro* digestibility of cooked rabbit meats were evaluated by TCA-soluble nitrogen release during digestion of pepsin and trypsin in simulated gastric fluid (Figure 3). The nitrogen release rate for boiled (B5, B15 and B40) and fried (F2, F4, F6) meats in pepsin section showed a similar trend during 30 to 60 min, but the differences appeared after 60 min. The nitrogen release rates for B15, B40 was higher than that of B5, F2, F4 and F6, especially after 120 min. The nitrogen release rate for longer duration frying (F6) was the lowest and the nitrogen release rate for B5, F2 and F4 were in between. Similar trend was observed during the trypsin digestion, but the nitrogen release rate for B5, B15 and B40, was found to be higher than that of F2 and F4. The nitrogen release rate for 6 min (F6) frying had the lowest values.

The *in vitro* digestibility of boiled and fried rabbit meat samples implied that, the boiled meats were easy to be digested than the fried ones. The longer boiling was found to be beneficial to the digestibility of meat protein while longer frying made the meat protein hard to be digested. The possible reason is that the frying leads to formation of heterocyclic aromatic amines in protein-rich

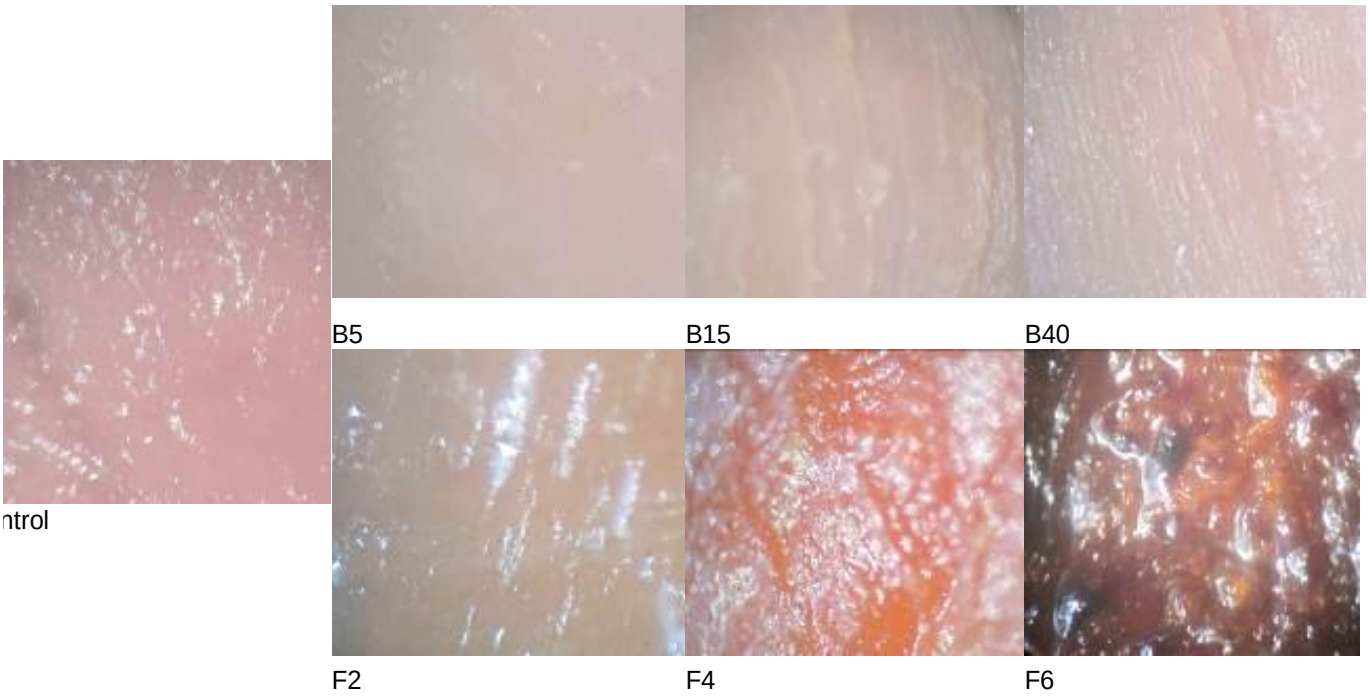

Figure 2. Effect of cooking on micro-surface of rabbit meat. "Control" is uncooked rabbit meat, B5, B15 and B40 represents meats boiled for 5, 15 and 40 min respectively; and F2, B4 and B6 represents meats fried for 2, 3 and 6 min, respectively.

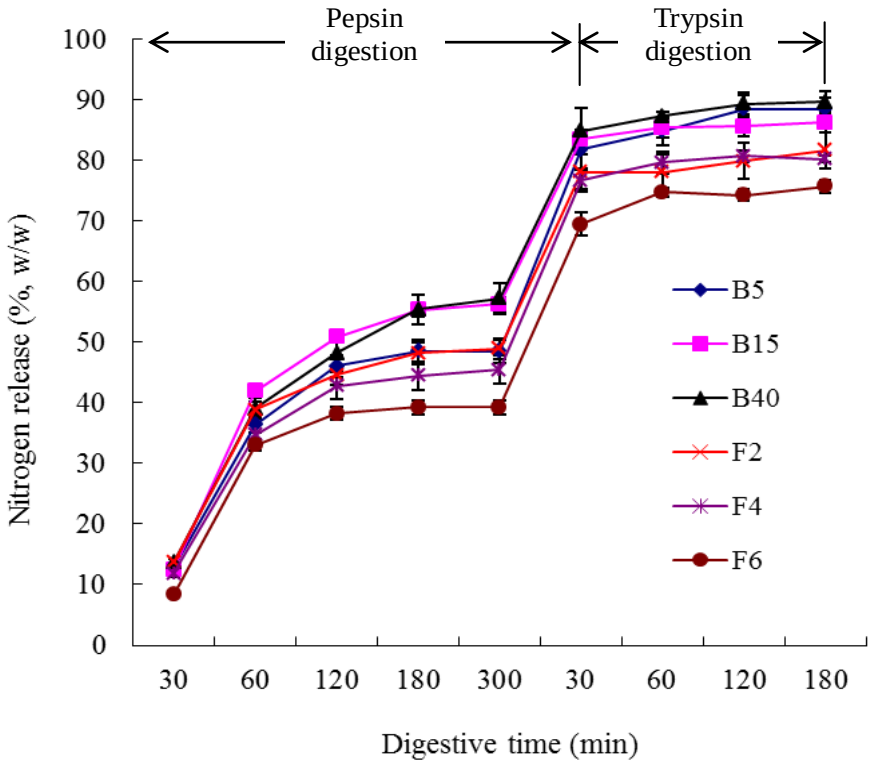

Figure 3. Effect of cooking on digestibility of rabbit meat *in vitro*. Means (n=3) without a common letter differ significantly (P<0.05); "Control" is uncooked rabbit meat, B5, B15 and B40 represents meats boiled for 5 min, 15 min, and 40 min respectively; and F2, B4 and B6 represents meats fried for 2, 3 and 6 min, respectively.

Table 2. Effect of cooking methods on amino acid of rabbit meat (mg/g protein).

Amino acid	B5	B15	B40	F2	F4	F6
Phenylalanine	9.012±0.018^{d}	11.414±0.020^{a}	10.427±0.131^{b}	9.096±0.006^{cd}	9.174±0.011^{c}	8.686±0.009^{e}
Alanine	18.922±0.067^{d}	21.145±0.034^{a}	21.227±0.094^{a}	18.157±0.075^{e}	19.698±0.094^{b}	19.300±0.057^{c}
Methionine	7.624±0.010^{c}	8.424±0.010^{b}	8.538±0.004^{a}	6.945±0.019^{e}	7.269±0.006^{d}	6.267±0.011^{f}
Proline	11.440±0.003^{d}	12.721±0.010^{a}	12.279±0.057^{c}	11.222±0.037^{e}	10.782±0.007^{f}	12.381±0.009^{b}
Glycine	14.736±0.160^{c}	16.257±0.086^{a}	16.106±0.075^{a}	15.311±0.094^{b}	15.323±0.037^{b}	14.640±0.057^{c}
Arginine	15.857±0.048^{c}	19.122±0.172^{a}	17.641±0.008^{b}	15.853±0.007^{c}	15.478±0.016^{d}	10.364±0.006^{e}
Lysine	20.829±0.058^{c}	25.043±0.064^{a}	22.817±0.075^{b}	20.066±0.031^{d}	16.667±0.008^{e}	12.453±0.004^{f}
Leucine	19.990±0.016^{c}	24.384±0.005^{a}	22.706±0.037^{b}	19.810±0.094^{d}	19.909±0.004^{c}	18.691±0.057^{e}
Tyrosine	8.554±0.005^{c}	10.584±0.052^{a}	9.632±0.094^{b}	8.550±0.057^{c}	8.277±0.037^{d}	7.877±0.024^{e}
Serine	10.913±0.144^{c}	12.267±0.052^{b}	12.478±0.094^{a}	10.278±0.094^{d}	9.184±0.057^{e}	3.318±0.112^{f}
Threonine	11.041±0.067^{c}	13.460±0.104^{a}	12.763±0.094^{b}	10.862±0.043^{d}	10.365±0.037^{e}	5.642±0.131^{f}
Glutamic acid	40.524±0.032^{d}	47.270±0.059^{a}	46.020±0.057^{b}	39.732±0.057^{e}	41.124±0.037^{c}	38.638±0.057^{f}
Aspartic acid	25.607±0.113^{b}	28.978±0.521^{a}	28.671±0.373^{a}	23.948±0.301^{c}	22.233±0.057^{d}	16.529±0.094^{e}
Isoleucine	8.521±0.006^{f}	11.630±0.031^{a}	9.732±0.013^{b}	9.097±0.075^{c}	8.836±0.004^{d}	8.686±0.006^{e}
Histidine	10.091±0.064^{e}	10.436±0.068^{d}	10.688±0.094^{d}	11.371±0.037^{c}	13.628±0.038^{a}	13.173±0.388^{b}
Valine	9.675±0.032^{e}	13.108±0.039^{a}	11.235±0.022^{b}	10.576±0.094^{c}	10.141±0.037^{d}	10.166±0.057^{d}
Cystine	0.609±0.010^{e}	0.582±0.012 ^{f}	1.567±0.008^{b}	0.674±0.009^{d}	1.197±0.011^{c}	2.126±0.008^{a}

Means (n=3) without a common letter differ significantly (P<0.05); B5, B15 and B40 represents meats boiled for 5, 15 and 40 min respectively; and F2, B4 and B6 represents meats fried for 2, 3 and 6 min, respectively.

foods such as meat and fish at temperatures mostly over 150°C (Oz et al., 2007), which are considered difficult to be decomposed and harmful as they are carcinogenic (Meng et al., 2009, Knize and Felton, 2005). Frying formed a hard dry layer on the surface of meat which prevented loss of internal nutrients of meat (Fillion and Henry, 1998) and also frying led to loss of meat protein (Yu et al., 1993). Harmful and hard to hydrolyzed chemicals, such as heterocyclic amines, formed during meat frying (Wong et al., 2012). All these facts could be cited as the possible reason for the slow nitrogen release rate of fried rabbit meats than those of the boiled meats.

Effect of heat cooking on amino acids content of rabbit meat

The mean amino acid contents of the boiled and fried rabbit meats are presented in Table 2 and the nutrition analysis of amino acids are presented in Table 3. All amino acids, except cystine, increased significantly (P<0.05) when rabbit meat was boiled for 15 min (B15) than boiled for 5 min (B5). The highest increase rate of 36.49% was observed for isoleucine, followed by valine with 35.48% increase. Meat boiled for 40 min also resulted in significant increase in the amino acid contents but at a lower rate when compared with 15 min boiling. These results are in acceptance with our findings on the digestibility of boiled meat which showed that the digestibility of B15 and B40 were easier than that of B5.

Most amino acids decreased significantly after frying for 4 and 6 min while frying for 2 min caused an insignificant (P>0.05) decrease in amino acids contents. Amino acids, which decreased during frying include proline, arginine, lysine, tyrosine, serine, threonine, aspartic acid, isoleucine and valine. Amino acids, lysine and serine had a higher decrease rate than other amino acids. Frying for 6 min caused a severe reduction in the amino acid content than the other two frying treatments, similar results were reported as four marine fishes commonly consumed in Nigeria (*Clupea harengus, Scomber scombrus, Trachurus trachurus* and *Urophycis tenuis*) were fried (Oluwaniyi et al., 2010). Though the boiling of meat for 15 and 40 min showed an increase in amino acid contents when compared with that of 5 min, meat boiled for 15 min contained more amino acids than the meat boiled for 40 min. Boiling resulted in denaturation of proteins in meat and the denatured meat proteins could be easily hydrolyzed (Adler-Nissen, 1976). When dissolved in hydrochloric acid for determination of amino acids, the meat boiled for longer time with high content of denatured proteins were easily hydrolyzed resulting in high amino acid contents than the meat boiled for shorter time. This is the reason for the increase in amino acid contents with the increase in boiling time. However, the prolonged boiling for 40 min resulted in more protein loss in meat and therefore the amino acid contents in meat boiled for 40 min were less than that of meat boiled for 15 min. This speculation agreed with that of Murphy et al. (2008), who reported that an endothermic transition at a temperature

Table 3. Effect of cooking methods on protein nutrition of rabbit meat.

Parameter	B5	B15	B40	F2	F4	F6
Total amino acids (mg/g, w/w)	243.9453±0.1403c	286.8250±0.8239a	274.5260±0.6699b	241.5478±0.1699d	239.2860±0.0980e	208.9370±0.0140f
Essential amino acid (% w/w)	35.5378±0.0621c	37.4667±0.0816a	35.7773±0.0281b	35.7906±0.0795b	34.4197±0.0289d	33.7858±0.1075e
*Chemical score	0.3043±0.0002e	0.4154±0.0011a	0.3476±0.0005b	0.3249±0.0027c	0.3156±0.0001d	0.2015±0.0047f
**Chemical score	0.6307±0.0040d	0.6523±0.0043c	0.6680±0.0059b	0.6998±0.0057a	0.6797±0.0003b	0.6269±0.0145d
*Essential amino acid index	0.3346±0.0005c	0.4041±0.0005a	0.3784±0.0009b	0.3354±0.0005c	0.3269±0.0003d	0.2871±0.0010e
**Essential amino acid index	0.6238±0.0008c	0.7535±0.0009a	0.7054±0.0017b	0.6253±0.0010c	0.6095±0.0006d	0.5353±0.0019e
Protein efficiency ratio	7.7091±0.0068 c	9.4911±0.0048a	8.8290±0.0227b	7.6279±0.0417d	7.7018±0.0022c	7.1908±0.0280e

Means (n=3) without a common letter differ significantly (P<0.05); B5, B15 and B40 represents meats boiled for 5, 15 and 40 min respectively; and F2, B4 and B6 represents meats fried for 2, 3 and 6 min respectively. *Based on amino acid requirements of school children (10-12 years); **Based on amino acid requirements of adults.

range of 59.6 to 68.4°C for collagen in chicken breast patties which resulted in an increase of soluble protein. The soluble protein dissolved in water caused decrease of protein content in chicken breast patties. Similar results were also obtained by Winegarden et al. (1952) for strips of collagenous material heated in distilled water.

The amino acid content of rabbit meat decreased with an increase in frying time. The possible reason is that frying led to formation of heterocyclic aromatic amines (Oz et al., 2007), and a hard and dry surface on the meat (Fillion and Henry, 1998), which made the meat proteins difficult to hydrolyze during amino acid analysis (Yu et al., 1993) and caused the meats fried for longer duration to have a low amino acid content. This result is consistent with that of longer frying time which resulted in low protein of cooked rabbit meat. Heterocyclic amines and polycyclic aromatic hydrocarbons formed during the frying cook, were found to contribute not only to the risk of developing colorectal tumors (Sinha et al., 1999), but also to lung adenocarcinoma (Butler et al., 2013).

The protein nutritional value of boiled and fried rabbit meats are shown in Table 3. The total and essential amino acid contents of boiled rabbit meats (B5, B15 and B40) are significantly (P<0.05) different from each other. The rabbit meat boiled for 15 min (B15) had the highest total and essential amino acid contents followed by meats boiled for 40 (B40) and 5 min (B5). The boiled rabbit meats (B5, B15 and B40) are also found to be significantly (P<0.05) different in the values of chemical score of school children (10-12 years) and adults, essential amino acid index of school children (10-12 years) and adults and the protein efficiency ratio. These results suggest that the boiling time is closely related to the nutritional facts of rabbit meat and there is an optimum time (15 min) to get maximum nutritional value in boiled meat. Boiling for a shorter or longer duration than the optimum time (15 min), resulted in decrease in the nutritional value.

When the protein nutritional value of fried meats were compared, it was found that the total amino acid contents in the meat fried for 4 min (F4) was significantly (P<0.05) lower than that for 2 min (F2), and the total amino acid content of F2 was significantly (P<0.05) lower than that for 6 min (F6). Similar significant (P<0.05) difference was discerned when the essential amino acid content, chemical score and essential amino acid index of F2, F4 and F6 were compared. Exceptionally, the protein efficiency ratio of F4 was found to be significantly (P<0.05) higher than that of F2, and the protein efficiency ratio of F6 was significantly (P<0.05) lower than that of both F2 and F4. The differences of protein nutritional value of F2, F4 and F6 implied that a proper and optimum frying time is essential to maintain the protein nutritional value of rabbit meat and frying for longer time resulted in a severe loss of protein nutritional value.

When comparing the protein nutritional value of boiled and fried rabbit meat, it can be found that the total amino acids, the essential amino acid index of both school children (10-12 years) and adults, and the protein efficiency ratio of boiled meats (B5, B15 and B40) are significantly (P<0.05) higher than that of fried meats (F2,F4 and F6). There is no similar significant (P>0.05) differences observed when the chemical score values of boiled and fried meats were compared. Except for chemical score of adults, all the protein nutritional value parameters of F6 is significantly (P<0.05) lower than B5, B15, B40, F2 and F4. This difference reiterated that frying rabbit meat for

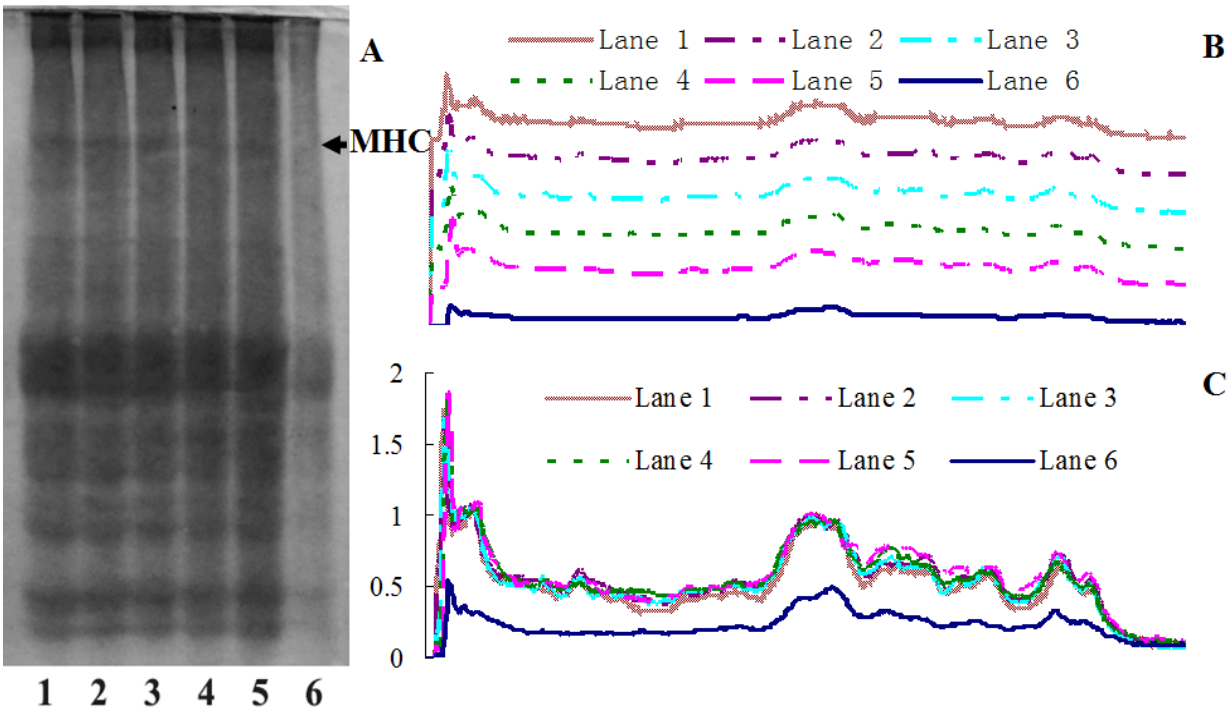

Figure 4. SDS-PAGE of the cooked rabbit meat. Lane 1, 2, 3 represents boiled 5, 15, 40 min, respectively; Lane 4, 5, 6 represents fried 2, 4 and 6 min, respectively; MHC is myosin heavy chains

longer time caused a severe nutrition loss. Beside the protein nutrition was decreased by frying, other investi-gations showed that frying process caused a signi-ficant alteration in the fatty acid profiles of hammour fish lipid and formation of harmful compounds in hammour fish fillets (Ganbi, 2011), heterocyclic aromatic amines were produced during pork frying, and their levels increased with increasing frying time and temperature (Zhang et al., 2013).

SDS-PAGE of cooked rabbit meat

In order to demonstrate the effect of boiling (B5, B15 and B40) and frying (F2, F4, F6) on protein pattern of rabbit muscle meat, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed. The results of SDS-PAGE analysis is shown in Figure 4A, along with the optical density (O.D) analysis of the gel (Figure 4B and C). From the Figure 4B and C, it was concluded that there is no difference between O.D values of B5, B15 and B40, but a notable difference is observed between the O.D curves of F2, F4 and F6. The O.D curve of F6 lies below the F2 and F4 (Figure 4 C) and also the O.D curve of F6 is different from all other heat treatments as shown in Figure 4 B.

The O.D values are proportional to the grey value of the bands in the electrophoresis image and a higher O.D value indicated larger grey value (Zhang et al., 2011). The difference of O.D in Figure 4B and C indicate that the protein pattern of the rabbit muscle meat was mildly affected by boiling for 5, 15 and 40 min and the protein pattern of rabbit muscle meat was moderately affected by frying for 2 and 4 min. But the protein pattern was seriously decreased by frying for 6 min. Boiling also resulted in the decrease of myosin heavy chains (MHC) (Figure 4A, B and C), which are polypeptides with a molecular mass of about 200 KDa (Sandri et al., 1999). This result agrees with the findings of Murphy and Marks (2000), who reported the decrease in the MHC of ground chicken breast after boiling.

Conclusions

The nutritional value of rabbit meat was significantly influenced when it was cooked by boiling or frying for different times. Boiling for 15 min enhanced the *in vitro* digestibility and nutritional value of rabbit meat, whereas boiling for 5 or 40 min led to their loss. Frying for 2 and 4 min helped to obtain an acceptable *in vitro* digestibility and nutritional value; even though the values were lower than that of boiled rabbit meat. The frying of rabbit meat for 6 min resulted in a severe impairment to the protein content, color, muscle surface structure, *in vitro* digestibility and protein nutrition. All these results strongly suggest that a proper boiling or frying duration is necessary to acquire a nutritious and high quality cooked rabbit meat.

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