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Levels of Heavy Metals in Local Milk and Cheese, and Phthalate Esters in Cheese by Settled Fulani Pastoralists in Ogun and Oyo States, Nigeria

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ABSTRACT

The quality of milk and cheese produced by Settled Fulani Pastoralists (SPFs) are of food safety concern. This study analysed heavy metals in milk and cheese, and phthalate esters in cheese products in Ogun and Oyo States Nigeria. Purposive sampling technique was used to select 55 equal products samples from 13 communities in the study areas. Milk and cheese samples were analysed for heavy metals (Cu, Ni, Zn, Cd, Cr, and Pb) using Atomic Absorption Spectrometer (AAS) after digestion with standard methods. Phthalate esters in cheese samples were extracted using sonication and analyzed using Gas Chromatography Flame Ionization Detector (GC-FID). The phthalate esters analyzed for in cheese are benzylbutyl phthalate (BBP), di-cyclohexylphthalate (DCHP), Di-2-ethylhexyl phthalate (DEHP), diethyl phthalate (DEP), diisobutyl phthalate (DiBP) and Dimethyl phthalate (DMP). The concentration of Cu, Ni and Zn were higher in cheese samples than in milk samples while levels of Cd and Cr were higher in milk than cheese. The mean concentration of phthalate esters in cheese were BBP (1.49 ± 0.94; 3.55 ± 4.78), DCHP (0.27 ± 0.15; 0.69 ± 1.09), DEHP(1.16 ± 1.93; 0.75 ± 1.06), DEP (1.45 ± 2.28; 1.81 ± 0.66), DiBP (3.27 ± 2.12; 5.75 ± 2.68) and DMP (1.06 ± 0.56;2.48 ± 1.08) for Ogun and Oyo States respectively. The study confirmed that heavy metals and phthalate esters were presented at high concentrations in milk and cheese samples analyzed.

Keywords: Food safety, milk, cheese, heavy metals, phthalate esters.

1.0 Introduction

Food safety is beyond the avoidance of food-borne biological pathogens, chemical toxicants and other hazards (FAO-UN, 2002). In particular, heavy metal and phthalate esters as contaminants in food present important food safety issues. Increasing complexity and length of food chain have led to an increase in contamination by heavy metals or other chemicals (Holdren and Ehrlich, 1974). These contaminants could be as a result of the production methods or materials used. Eating habits of consumers have also contributed to emerging and reoccurring food safety problems (Wilcock *et al*., 2004; Sirieix *et al*., 2007; Marvin *et al*., 2009). Milk is one of the sources of animal protein and it is mostly consumed in Nigeria as local yoghurt, sour milk, boiled or fried cheese, and powdered, condensed and or evaporated milk (Ganguly *et al*., 1999). Although many Nigerians consume milk which is industrially processed in different forms, the consumption of locally produced milk by Settled Fulani Pastoralists (SFPs) is of economic significance. The SFPs are local dairy producers that migrate in search of an ideal condition for raising their herds and means of survival.

The SFPs women especially take milk production, processing and marketing as a means of livelihood (Fabusoro and Oyegbami, 2009). However, several studies have proven that local production of milk

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by SFPs is carried out under unhygienic conditions that influence contaminations (Omotayo *et al.*, 2013). Phthalates esters (PEs) are a family of compounds with a common chemical structure. They are not chemically bound together and so can be released into the environment causing toxicity or contamination to products during production. Polyvinylchloride (PVC) consumer products, food and beverage packaging, and children's toys are made from phthalate esters compounds. These release the pollutants into the environment to influence clinical health problems and toxicities in human. The threats include reproductive and developmental issues (Lovekamp-Swan and Davis, 2003; Ono *et al.*, 2004) and thyroid atrophy in rodents or human (Meeker *et al.*, 2007). They can be released from soft plastics because they are used in plastic industries as plasticizers to impart flexibility to plastics (Rahman and Brazel, 2004).

Packaging of food and beverages is usually done in common plastic. Thermoplastic is the plastic material polymer melted with industrial enhancers and then re-solidified without degrading. Acceptable usage of plastic products in food and beverage industries had been recommended to influence high risk of exposure to phthalate threats (Saad *et al.*, 2015). Plasticiders (phthalate esters) are added to the raw materials of plastic at varying proportions to meet industrial properties and benzene di-carboxylic acid esters with diverse levels of toxicity. They degrade slowly through hydrolysis process of an ester bond to analogous mono-ester, hydroxylation of the alkyl moiety and lastly, mineralization (Saad, 2016). The PEs are known to be harmful to the human body when ingested and accumulated overtime, thus causing various ailment. Some PEs are classified as endocrine disruptive chemicals. Consumption and accumulation of phthalate esters in the human body may result in diseases such as ulcerative stomatitis, blue gingival (lead line), abnormalities in or low sperm number or count, decrease in sexual drive, impotence and sterility, abnormal ovarian cycle, menstrual disorders and spontaneous abortion (Gallinger and Nguyen, 2013). Organic and inorganic contamination remains an important issue in public health; either of the two is potentially harmful to the human body through ingestion, hence causing various diseases or death under severe conditions. Despite several laws guiding the production of food, local producers such as the SFPs and consumers may not be aware of the health implications of unhygienic food and the conditions of preparation of the food derivatives and other products: milk and cheese which this study considered. The study thus determined the safety of milk and cheese produced by the SFPs considering heavy metals and PEs contamination in selected communities of Ogun and Oyo States, Nigeria.

2.0 Materials and Method

2.1 Sample collection

Raw milk and cheese samples were collected in 13 communities from some local government areas of Ogun and Oyo States. A total of 55 milk and 55 cheese samples produced by SFPs were collected from 8 SFPs settlement in Ogun State and 5 SFPs settlements in Oyo state (Table 1).

The milk and cheese collected were transported to the laboratory in ice chest. The cheese samples were collected in glass sample bottles to prevent any form of contamination. Approximately 5 g of cheese was weighed and 5 mL of the raw milk sample was measured and transferred into different 12 mL quartz vessels. The samples were subjected to heat at a very low temperature (30^{o}C) in a cooling bath for 20 minutes. The dried samples were then transferred into muffle furnace to ash at 550^{o}C for 30 minutes. The obtained ash was dissolved in 10 mL of HNO_3 and heated on a steam bath to complete the dissolution. The dissolved ash was filtered through Whitman filter paper (No. 42). The filtrate was diluted with 100 mL deionized water and later transferred into polypropylene tubes. Determination of six selected heavy metals (Pb, Zn, Ni, Cr, Cu and Cd) concentration was determined

Table 1: Sampling locations

States	LGA	Communities	No. of processor
OGUN	Yewa North	Igborin	15
		Isa-Ope	5
		Awela	1
	Odeda	Okiki	2
		Malakai	2
		Budo Aiyetoro	4
		Arakanka	3
		Budo	2
OYO	Atiba	Agba	1
	Atigbo	Alaga	1
	Itesiwaju	Ipapo	4
		Okaka	5
	Saki West	Oja-Agbe	15
Total	6	13	55

*Digestion of milk and cheese sample for heavy metals analysis.

using Atomic Absorption Spectrophotometer (AAS) (Buck Scientific, USA, 2010) (Buck 210, VGP) at the University of Ibadan Central Laboratory, Ibadan, Nigeria.

2.2 *Preparation of samples for phthalate esters analysis*

The cheese samples collected were transported to the laboratory on ice chest. All the laboratory glass wares used were washed and rinsed with heptane and later oven dried. Using analytical weighing balance, 5 g of cheese sample was weighed in a pre-cleaned test tube. Ten (10) mL of heptane was added to the sample and centrifuged at 10 mm/1500 r/min. After 10 minutes, the solution was filtered using filter paper containing anhydrous sodium salt. The filtrate was decanted into a sterilized conical flask. The procedure was repeated twice and the filtrates were combined in a conical flask. The flask was covered with perforated aluminum foil for the sample to dry in the fume cupboard. The dried sample was re-diluted with 1 m of heptane and the solution was transferred into vials with pasteurized pipette. The samples were analyzed at Hydrochrom Analytical Laboratory, Lagos, Nigeria using Gas Chromatography Flame Ionizing Detector (GC-FID) using prescribed method (Olujimi, 2012).

2.3 *Statistical analysis*

Data obtained were subjected to descriptive analyses (mean, standard deviation and minimum and maximum) using SPSS version 17.

3.0 Results and Discussion

3.1 *Levels of heavy metals in milk and cheese samples*

3.1.1 Cadmium

The levels of Cd (ppm) ranged from 0.14 ± 0.07 in Ogun State cheese samples to 0.23 ± 0.14 in Ogun State milk samples (Table 2). In Ogun and Oyo States respectively, the minimum and maximum values of Cd (ppm) were 0.51 to 3.11 and 1.92 to 2.66 in milk samples, while 0.69 to 7.63 and 2.10 to 3.88 in cheese samples. The mean values were higher than EU standard (0.05 ppm) (Figure 1). The level of Cd in the milk samples was higher than in cheese. This could be ascribed to the coagulant used during cheese processing. The level of Cd in the milk samples was higher than in cheese samples. The mean concentrations of Cd in the milk samples in the two states were not different: (0.23 ppm). The presence of Cd may be due to the contamination of the soil, fodder that grows on the soil and water consumed by the animals. Accumulation of Cd in the human body leads to hypertension, gastroenteritis, pulmonary oedema, severe pain, softy bones and finally death (Faust and Aly, 1981; Peter, 1993; Mansour, 1999). The mean values in this study exceeded the previous study's value: 0.0.7 ± 0.02 ppm in milk, but conformed to the value: 0.19 ± 0.01 ppm in cheese (Aniello *et al.*, 2006).

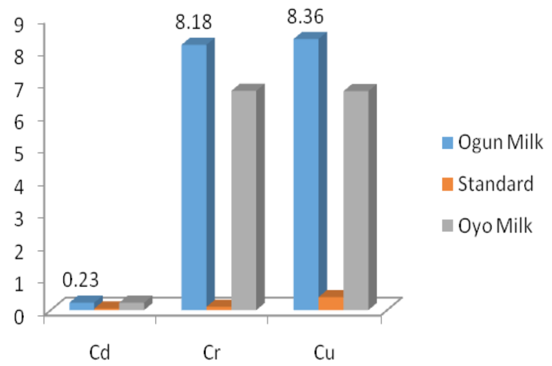

Fig. 1: Cd, Cr and Cu in milk samples against EU standard

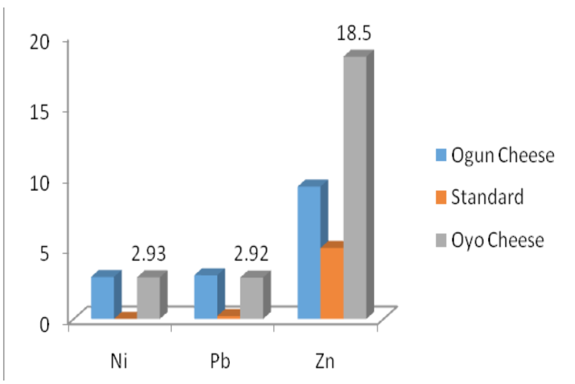

Fig. 2: Ni, Pb and Zn in milk samples against EU standard

3.1.2 Chromium

The levels of Cr (ppm) were detected to range from 6.04 ± 3.31 in Ogun State cheese samples to 8.18 ± 2.57 in Ogun State milk samples. The level of Cr in the milk samples was higher in milk than in cheese samples. In Ogun and Oyo States respectively, the minimum and maximum values (ppm) of Cr were 0.06 to 0.49 and 0.14 to 0.40 in milk samples, while 0.05 to 0.25 and 0.04 to 0.44 in cheese samples. The mean level of Cr in both milk and cheese samples was higher than the EU stipulated standard (0.10 ppm) (Table 2). The mean values in this study exceeded the previous study's values: 0.14 ± 0.11in milk and 0.46 ± 0.42 in cheese (Aniello et al., 2006). The mean level of Cr in both milk and cheese was higher than EU stipulated standard (Figures 1 and 3).

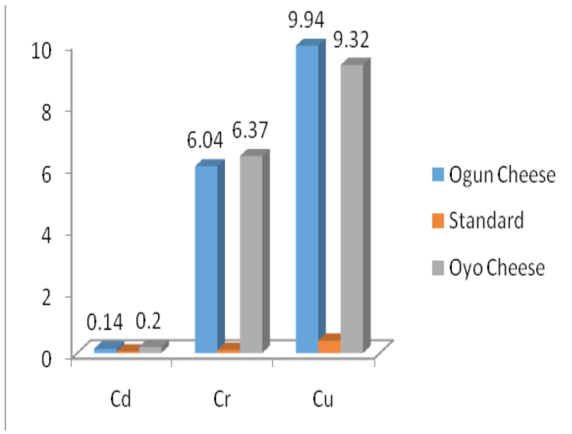

Fig. 3: Cd, Cr and Cu in cheese samples against EU standard

3.1.3 Copper

The level of Cu (ppm) ranged 6.75 ± 3.37 in Oyo State milk to 9.94 ± 2.42 in Ogun State cheese samples. The Cu concentration ranged from 4.94 to 10.91, 3.42 to 8.80 in milk and 0.82 to 9.63, 3.42 to 8.19 in cheese samples for Ogun and Oyo States respectively. The mean levels were higher than the EU stipulated standard (0.40 ppm) (Figures 1 and 3). The levels of Cu in milk samples were higher than in cheese samples. The high concentration of Cu could be traced to uncontrolled mineral supplements given to the cattle by the pastoralists (Ogundiran *et al.*, 2012). Copper is known to have a powerful emetic action and a repulsive taste. The toxic effect of excess Cu in human body can result in bloody diarrhoea, hypotension, jaundice and nausea. This conformed to the finding of Ogundiran *et al.* (2012) that Cu can be toxic to humans when consumed in excess and the presence of Cu in milk sample could be attributed to cows being allowed to graze around contaminated site.

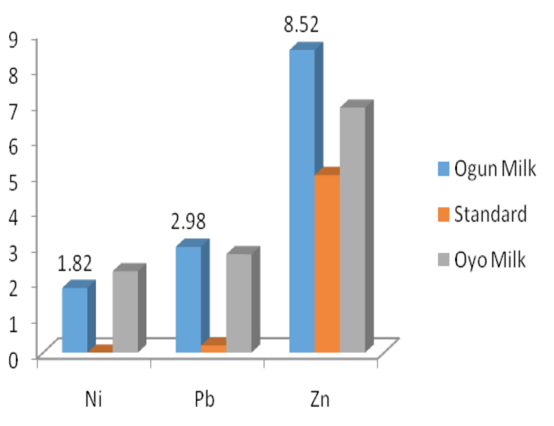

Fig. 4: Ni, Pb and Zn in cheese samples against EU standard

3.1.4 Nickel

The levels of Ni (ppm) were detected to range from 1.82 ± 0.92 in Ogun State milk samples to 2.96 ± 2.37 in Ogun State cheese samples. In Ogun and Oyo States respectively, the minimum and maximum values (ppm) of Ni were 0.72 to 7.70 and 0.83 to 6.66 in milk samples, while 0.81 to 8.33 and 1.65 to 4.74 in cheese samples. The mean level of Ni in the cheese samples was higher than in milk samples (Table 2). The level of Ni in both milk and cheese samples was higher than the EU stipulated standard (< 0.10 ppm).

3.1.5 Lead

Lead concentration (ppm) was detected to range from 2.77 ± 2.34 in Oyo State milk samples to 3.08 ± 2.32 in Ogun State cheese samples. In Ogun and Oyo States respectively, the minimum and maximum values (ppm) of Pb were 1.98 to 13.04 and 1.86 to 10.03 in milk samples, while 5.83 to 12.58 and 4.56 to 12.08 in cheese samples. The mean level was found to be higher than the EU stated standard (0.20 ppm) (Table 2). The presence of Pb content in the samples could be linked to its high affinity to casein which is one of the contents found in milk. It can also be attributed to possible contamination from pastoralists and their environment. The result corroborated the study of Aniello *et al.* (2006) that Pb in milk can be a consequence of contamination during the milking and processing of cheese. The mean values exceeded the previous study's values (0.47 ± 0.37) in cheese samples (Aniello *et al.*, 2006). Ingestion of high levels of Pb or its excessive dose in the body system is capable of causing ulcerative stomatitis, a blue gingival (lead line), abnormalities in sperm number or low sperm count, decreases in sexual drive, impotence and sterility, abnormal ovarian cycle, menstrual disorders and spontaneous abortion (Sharara *et al.*, 1998). This was attributed to low rate of lead elimination when accumulated in the body (Ibels and Pollock, 1986; Papajov and Hermanov, 1990; Yüzbas *et al.*, 2003).

3.1.6 Zinc

The mean concentrations of Zn (ppm) were detected to be 8.52 ± 2.94 and 6.90 ± 2.39 in milk samples, while 9.33 ± 3.39 and 18.50 ± 8.37 in cheese samples of Ogun and Oyo States respectively. In Ogun and Oyo States respectively, the minimum and maximum values (ppm) of Zn were 5.42 to 13.33 and 5.1 to 10.92 in milk samples, while 3.21 to 13.05 and 12.7 to 32.90 in cheese samples. The mean levels were higher than the EU Standard (5.00 ppm) (Table 2). The Zn concentration was higher in Oyo State cheese sample than in Ogun State cheese samples. The level of Zn in the milk samples was higher than in cheese samples. Furthermore, the highest value of Zn (18.5 ppm > EU standard: 5.0) detected in all the samples corroborated to the previous studies (Triphathi *et al.*, 1999; Martino *et al.*, 2001; Licata *et al.,* 2004). The high concentration of Zn observed

Table 2: Levels (ppm) of heavy metals in milk and cheese

			Milk			
State	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
Ogun Milk	0.23 ± 0.14	8.18 ± 2.57	8.36 ± 4.12	1.82 ± 0.92	2.98 ± 2.43	8.52 ± 2.94
Min	0.51	0.01	4.94	0.72	1.98	5.42
Max	3.11	0.49	10.91	7.70	13.04	13.33
Oyo Milk	0.23 ± 0.11	6.76 ± 2.20	6.75 ± 3.37	2.29 ± 0.29	2.77 ± 2.34	6.90 ± 2.39
Min	1.92	0.14	3.42	0.83	1.86	5.1
Max	2.66	0.40	8.80	6.66	10.03	10.92
			Cheese			
Ogun	0.14 ± 0.07	6.04 ± 3.31	9.94 ± 2.42	2.96 ± 2.37	3.08 ± 2.32	9.33 ± 3.39
Min	0.69	0.05	0.82	0.81	5.83	3.21
Max	7.63	0.25	9.63	8.33	12.58	13.10
Oyo	0.20 ± 0.15	6.37 ± 1.82	9.32 ± 3.01	2.93 ± 0.68	2.92 ± 1.38	18.5 ± 8.37
Min	2.1	0.04	3.42	1.65	4.56	12.66
Max	3.88	0.44	8.19	4.74	12.08	32.90
Standard	0.05	0.10	0.40	≤0.10	0.20	5.00

may be attributed to uncontrolled administration of mineral supplements by the pastoralists to meet the Zn requirements of the animals. It could also be attributed to the environment where the animal grazed or the sites where the Sodom apple plant (used as coagulant in cheese) grows or is found. The environmental factors can be related to improper disposal of rubber materials. This conformed to the findings of Ogundiran *et al.* (2012) that residues of burnt tyres are known to contain high level of zinc. High concentration of Zn had been reported (LeMone, 1999) to have negative implication on human health when consumed regularly.

Generally, detectable residual concentrations of Cd, Cr, Cu, Ni, Pb and Zn were observed in all milk and cheese samples collected in both states. There was an obvious variation in all the levels of heavy metals detected except Pb. The results showed that all the heavy metals detected were above the European Union heavy metals intake permissible limits. Levels of Cu, Ni and Zn were higher in cheese samples than in milk samples; levels of Cd and Cr were higher in milk samples than in cheese samples, while levels of Pb uniformly varied in both samples. The mean concentrations of Zn, Cu and Cr were higher than Pb, Ni and Cd in both samples. This could be attributed to the difference in geographical locations, soil types, water and other environmental factors. This corroborated the findings of Ogundiran *et al.* (2012) who recorded high levels of Pb in milk samples produced in animals raised around contaminated sites.

The residue concentrations of the heavy metals (Cd, Cr, Cu, Ni, Pb and Zn) in raw cow milk and cheese samples are presented in Figures 1 to 4. Heavy metal contamination raises environmental concerns which can be potentially harmful to humans. They are known to have a multitude of toxic effects such as acute syndrome and neurotoxic effects (that ultimately cause diseases in the brain, kidney, and skin cancer). Agriculture constitutes are one of the very important sources of heavy metals pollutants and are impurities in fertilizers, pesticides and sewage sludge. Increased industrialization and agricultural activities had been accompanied by extraction and distribution of mineral substances

(Wahlberg *et al.*, 2001). Heavy metal impurities in milk and milk foodstuffs influence adverse effects on public health and the incidence of different illnesses and injuries are high owing to the extensive consumption of the products. Inputs with either animal or plant resources need be monitored before use. Food packaging and preparation materials must not be harmful to human health (Mahmoudi *et al.*, 2017).

3.2 Levels of phthalates esters in cheese samples

Phthalate esters (PEs) detected from the analysed cheese samples were benzylbutyl phthalate (BBP), di-cyclohexyl-phthalate (DCHP), di-2-ethylhexyl phthalate (DEHP), diethyl phthalate (DEP), diisobutyl phthalate (DiBP) and dimethyl phthalate (DMP). From Table 3, the mean concentrations of phthalate esters in the samples collected from Ogun and Oyo States respectively are BBP (1.49 ± 0.94; 3.55 ± 4.78), DCHP (0.27 ± 0.15; 0.69± 1.09), DEHP (1.16 ± 1.93; 0.75 ± 1.06), DEP (1.45 ± 2.28, 1.81 ± 0.66), DiBP (3.27 ± 2.12; 5.75 ± 2.68) and DMP (1.06 ± 0.56; 2.48 ± 1.08). The phthalate esters with the maximum residual value in Ogun State was DEP (7.06) while BBP (11.82) in Oyo State. Generally, the PEs concentration were higher in Oyo State samples than those of Ogun State.

Table 3: Phthalate esters in cheese samples

Phthalate esters	Cheese samples in Ogun State				Cheese samples in Oyo State			
	Standard	Min	Max	Mean ± SD	Standard	Min	Max	Mean ± SD
BBP	0.20	0.10	3.10	1.49 ± 0.94	0.20	0.30	11.82	3.55 ± 4.78
DCHP	< 0.05	0.10	0.56	0.27 ± 0.15	< 0.05	0.10	2.62	0.67 ± 1.09
DEHP	0.02	0.14	5.90	1.16 ± 1.93	0.02	0.10	2.62	0.75 ± 1.06
DEP	0.80	0.18	7.02	1.45 ± 2.28	0.80	0.07	2.61	1.81 ± 0.66
DiBP	0.10	0.71	6.29	3.27 ± 2.12	0.10	2.20	8.40	5.75 ± 2.68
DMP	< 0.01	0.38	2.03	1.06 ± 0.56	< 0.01	1.74	4.35	2.48 ± 1.08

Previous researches had traced DEHP, BBP, DiBP and DEP to plasticizers in food-packaging and food-contact materials (Bradley *et al.*, 2013; Robertson, 2013). All the phthalate esters detected in the cheese samples were above the EU permissible limits. Levels of detection DiBP > BBP > DMP in this study conformed to the order detected by Schecter *et al.* (2013) but contrasted to the order DEHP > DEP which were detected to be greater DiBP, BBP and DMP. Both DMP and DEP are PAEs with low molecular weight used for manufacturing of cosmetics and personal care products, while DEHP and BBP are classified as PAEs with a larger molecular weight commonly used in polymer industries as plasticizers to improve flexibility of handling properties.

The levels of DEHP in this study were lower than the levels recorded in previous work in varied food samples, wherein the highest concentration (35 ppm, fat basis) was found in cheese (Staples *et al.*, 2000; Anderson *et al.*, 2001; Peijnenburg and Struijs, 2006).

4.0 Conclusion

The study concluded that SFPs' milk and cheese are not safe for public consumption. Phthalate congeners with -butyl- constituents (diisobutyl phthalate (DiBP) and benzylbutyl phthalate (BBP)) were observed to have the highest mean values in both categories of samples. Continuous consumption implies possible bioaccumulation of both heavy metals and phthalate esters. Low public awareness of food safety and indifferent health belief would worsen the adverse health effects. As the livelihoods of SFPs women depend on these products, the study recommended the need for food regulatory agents' interventions to upgrade milk and cheese processing methods for quality and safe products.

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