A REPORT ON

EVALUATION OF MILK PRODUCTS DERIVED FROM NAK/CHAURI OF SOLUKHUMBU DISTRICT OF NEPAL FOR POTENTIALLY BENEFICIAL CHARACTERISTICS.

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ACRONYMS

ALA	Alfa Linoleic Acid
AOAC	Association for Official Analytical Chemists
CLA	Conjugated Linoleic Acid
DHA	Docosahexaenoic Acid
EPA	Eicosapentaenoic Acid
FAO	Food and Agriculture Organization of the United Nations
FID	Flame Ionization Detector
GC	Gas Chromatography
Kg	Kilo gram
MFGM	Milk Fat Globule Membranes
MUFA	Monounsaturated Fatty Acid
PUFA	Polyunsaturated Fatty Acid
SD	Standard Deviation
SFA	Saturated Fatty Acid
WHO	World Health Organization
β-CN	Beta-Casein

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GLOSSARIES

Chauri: *Chauri* is the generic word in Nepali language to describe an animal, either of pure breed or cross bred, that has characteristic long hair, long and spiky horns, and a thick hair bunch at the tip of the tail. Chauri is farmed at high altitude and the Himalayan region for milk production and transportation of goods.

Yak: Yak is word of Tibetan or Sherpa language to describe the bull of the pure breed Chauri.

Nak: It is also a word from Tibetan or Sherpa language used to describe the milking female of pure breed Chauri

Yak and Nak have been domesticated by human for hundreds of years. However, some wild Yak and Nak are also found in the region.

Jholang: Jholang is the word in Tibetan/Sherpa Language used to describe the bull of the hump less Tibetan cattle (*Bos taurus*).

Aule Gai: The female of the local hill cattle or zebu cattle (*Bos indicus*), which has hump.

Jhom: The cross bred milking Chauri produced by cross breeding between Nak and Jholang or Yak and Aule Gai. There are two types of Jhom, namely Dimjo Jhom and Urang Jhom

Dimjo Jhom: The cross bred milking Chauri produced by cross breeding between Nak (Pure breed female Chauri) and Jholang (Bull of the hump less Tibetan cattle) is called Dimjo Jhom or Dimjo Chauri. Dimjo Jhom is comparatively larger in body size, adaptable to high altitudes, and also give more milk. However, Dimjo Jhom has some disadvantages as it is relatively passive, tends to graze only at easier places, has higher temptation to grazing for longer period of time, does not return home/farm at regular time and also less responsive to the words or sounds that are used by the herders to control the Chauri.

Sherkham: Sherkham is a Chauri milk product produced by coagulation of buttermilk obtained after churning of Chauri cream or milk. Buttermilk is boiled and coagulated by adding acidic whey saved from earlier days. The coagulated protein separated by straining through a cotton cloth is called Sherkham. Whey is mostly discarded except

some whey is saved for coming days to coagulate buttermilk. Fresh Sherkham is sundried into dry powder and stored airtight.

Urang Jhom: The cross bred milking Chauri produced by cross breeding between Yak (Pure breed Chauri Bull) and Aule Gai (Female zebu cattle) is called Urang Jhom or Urang Chauri. Urang Chauri is farmed at lower altitudes. Urang Jhom gives relatively less quantity of milk; however, milk is thicker due to higher total solid and fat contents. Compared to Dimjo Jhom, Urang Jhom is more active, grazes at difficult places such as steep slopes, returns to the farm at regular time, likes to follow the herders and obeys the words or sounds that are used by the herders to control the Chauri. For these reasons, herders prefer Urang Chauri over the Dimjo Chauri.

Zhopkyo: The cross bred male chauri, regardless of the types of parents used, are called Zhopkyo. Zhopkyo are sterile.

EXECUTIVE SUMMARY

This report is aimed to highlight the beneficial properties of Chauri milk by reviewing the relevant literatures and by conducting laboratory tests of Chauri milk and milk products. Several published materials on Yak/Chauri Milk had been reviewed and adequate information was collected in relation to beneficial properties of Chauri milk. Review of literature suggested that Chauri milk is considered superior to cow milk due to availability of higher quality proteins such as β-casein. Chauri milk also found to contain Glycosylation-dependent cell adhesion molecule 1 (GlyCAM1), CD59 molecule and lactadherin, which are believed to have antimicrobial and antitumor effects. From review of literatures, it was also found that milk from Chauri contains compounds named exosomes that help in tolerance of hypoxia (low oxygen concentration). Some hydrolyzates of Chauri milk protein showed antioxidant properties.

From the literature review, it was also found that Chauri milk contain higher concentration of Omega-6 and Omega-3 fatty acids, Polyunsaturated fatty acids (PUFA), which benefit by preventing heart diseases, improving brain functions, blood clotting and muscle strength. Literatures also showed that Chauri milk fat contains Conjugated linoleic acid (CLA) as high as 3 times the concentration found in cow milk fat. CLA is considered highly important due to its anticancer activity against various types of cancers indicating that Chauri milk fat helps to combat cancers. Chauri farmed using open pastures at higher altitude consume beneficial herbs, which can also be traced to milk that further increases the beneficial properties of milk.

Most of the information from the literatures are obtained by reviewing researches and studied conducted in other countries. During the literature review, studies supported by reliable laboratory analyses to support the beneficial properties of Chauri milk in Nepal were not found. Therefore, samples of Chauri milk, and milk products were collected from Solukhumbu District and analysed for detailed fatty acid profiling using the most reliable Gas Chromatography method in an internationally accredited laboratory in Nepal.

Laboratory analysis of samples showed that Fats from Chauri milk contains higher concentration of Omega-3 and Omega-6 fatty acids, higher concentration of

polyunsaturated fatty acids (PUFA), and higher concentration of conjugated linoleic acid (CLA). Chauri milk produced during summer contains these beneficial fatty acids even in higher concentration than in winter.

Few fatty acids found in cow milk fat were not found in Chauri milk fat, and few fatty acids found in Chauri Milk were not found in cow milk fat. The fatty acids, which are specifically found in Cow milk fat or Chauri milk fats may potentially be used as markers to determine adulteration of Chauri milk with cow milk or vice versa.

Based on this study following key finding has been summarized:

> ***Chauri milk fat has anticancer properties due to the presence of higher concentration of Conjugated Linoleic Acid (CLA). Chauri milk obtained during summer season is better compared to winter milk. Chauri milk fat also contains Omega-6 and Omega-3 fatty acids, which help preventing heart diseases, and improving brain functions, blood clotting and muscle strength.***

1. INTRODUCTION

1.1. Chauri

Pure Chauri (*Poephagus grunniens* or *Bos grunniens*) is a cattle native to alpine and subalpine regions at altitudes of 2000-5000 meters along the Himalayan region of Nepal, Bhutan, China (Tibet), Mongolia and India. For the Himalayan people, Chauri are the prime source of milk, meat and wool. Chauri are also used as load animals for transportation of goods. Wild yak is actually the progenitor of domesticated Yak, whereas the domesticated Yak generally called Chauri in Nepal, is a progeny of Wild Yak produced by crossbreeding with hill cattle or Yak-hill cattle cross breeds (Rameshwar Singh Pande, 2007; Y Ma, 2013). A report of 2007 had estimated world population of Yak to be 14 million, out of which approx. 0.2 million Yak and its crosses were estimated in Nepal (Rameshwar Singh Pande, 2007). In China, at least 12 Yak breeds have been reported that belong to mainly two types: Qinghai-Tibet Plateau type and Henduan Alpine type(Y Ma, 2013). Yak is raised in 22 Himalayan districts of Nepal along the Tibetan boarder.

Even though pure Yak breed had also been domesticated by the people native to the higher Himalayan regions, cross breeding of Yak is popular in traditional farming practice mainly due to higher performances of the cross breeds (more milk and meat), and better adaptability to wider range of altitudes. For Yak cross breeding, *Bus grunniens*, pure Yak breed, is bred with either a) *Bos taurus*, which is small hump less cattle (also known as Kirkho) originally native to Tibet, or b) *Bos indicus*, which is Zebu cattle (Also known as Aule cattle) characterized with large hump and adapted to low to middle altitudes (Dong et al., 2009; Mohan Kharel, 1995; Mohan Kharel et al., 2005).

Chauri is a term generally used in Nepal to describe cross bred Yak. Broadly, there are two types of Chauri, namely a) Urang Chauri and b) Dimjo Chauri. When a pure male Yak and a female Zebu cattle are cross bred, the hybrid offspring is called Urang Chauri. Whereas, when a pure Yak either male or female and Kirkho cattle are cross-bred, the hybrid offspring is called Dimjo Chauri. The male offspring, either Dimjo or Urang, produced by cross breeding of Yak with other cattle species is called Zhopkyo, which is always sterile. Female offspring, either Dimjo or Urang are also called Jom, which are

fertile. Due to the sterility of the male off spring (Zhopkyo), reciprocal cross breeding withing the hybrids is impossible (Todd M. Silk et al., 2006).

Dimjo and Urang cross breeds have their own advantages and disadvantages. Herders at the higher altitudes prefer Dimjo, whereas, herders at the lower altitudes prefer Urang. Normally, Dimjo Chauri are larger in size and give more milk compared to Urang Chauri, but Urang Chauri have better adaptability to wider range of climatic condition than Dimjo Chauri(Mohan Kharel, 1995; Mohan Kharel et al., 2005).

1.2. General Fallacies about Chauri

a) **Yak milk**

"Yak" is the word of Tibetan and Sherpa languages that was later adopted in English. In English, the word Yak describes the animal regardless of whether it is male or female and of pure breed or cross bred. The Chauri herders in Nepal are mostly the Sherpa people, for whom Yak means only the bull of the animal. Therefore, the term "Yak milk" does not make sense to the Chauri herders, instead they feel difficulty when people say "Yak milk". For example, when we had asked a Chauri keeper woman of Lower Fera village near Salleri, Solukhumbu, that how much milk her *Yak* gives, she felt awkward and shy and tried to avoid answering. This is because, in her understanding, we were asking that how much milk the bull gives! Therefore, for the Sherpa people, to say Yak milk is false, and to call it Nak milk is true (Source: personal communication with the Yak and Chauri herders of Solukhumbu district).

As Sherpa people are native to Nepal, to call it Yak milk or Yak milk product is actually wrong. It should be proper to call it Chauri milk or Chauri milk product, or at least Nak milk or Nak milk products.

b) **Short Lives of Calves from Dimjo or Urang Chauri**

Dimjo and Urang are the female hybrid Chauri, which are bred for milk production. The adult Dimjo or Urang Chauri are inseminated by Yak, Jholang or bull of Aule cattle, whatever is available. This is because most of the calves of Urang or Dimjo are useless regardless of their father. Calves of Dimjo or Urang are born with shorter lives, generally

of only up to 2 months. After the death of the young calves, the Chauri continue giving milk till the end of the lactation period. Most of the people, who do not know about this fate of young calves of the hybrid Chauris, think that the Chauri herders intentionally starve the calves to death. The Chauri herders are found to be hurt by such false understanding (Source: Personal communications with the Yak and Chauri herders of Solukhumbu district).

1.3. Chauri as a Milk Animal

Yak/Chauri are kept in the Himalayan region mainly for three purposes: a) milk, b) meat and c) pack animal. Normally Zhopkyo, the sterile male Chauri, is used as pack animal. Pure Yak breeds have also been raised since many years by the Himalayan people for all the three purposes, however, these performances are improved in Dimjo and Urang Chauri.

Difference in milk yield by Yak and Chauri in Nepal has been reported. According to one report, milk production of pure Nak (female of pure Yak cattle is called Nak) during the lactation period of average 180 days is approximately 720 kg compared to 1300 kg and 1700 kg of Urang chauri and Dimjo Chauri respectively. Nak, Dimjo Chauri and Urang Chauri all have their lactation length of approximately 6 months (Mohan Kharel et al., 2005). Another report has suggested milk yield of Nak, Dimjo and Urang Chauri as 200, 300-540, and 300-540 liter during a lactation period of 167, 120-180 and 120-180 days, respectively. Normally, Nak is milked half while leaving half of the udder for the calves. Considering this, total yield of milk may actually be double than reported (Todd M. Silk et al., 2006). In China, artificial insemination of Yak with high yielding breed such as Holstein has resulted in almost three-fold increase in milk yield compared to the milk yield by pure Yak breed (Todd M. Silk et al., 2006). In contrary to this, cross breeding of Yak with Holestein-Friesian, Jersey and Brown Swills cattle resulted in disappointing performance of the hybrids in terms of milk production suggesting that breeding of pure Yak with local breeds of Himalayan and Zebu cattle is the best option to enhance performance of ChaurI (FAO, 2003). Reports on milking performance of Nak/Chauri in Nepal has been summarized in Table 1.

Table 1. Milking performance of Nak/Chauri in Nepal.

Performance parameters	Nak	Dimjo Chauri	Urang Chauri	Reference
Lactation Period (Days)	180	260	260	(FAO, 2003)
Milk yield (kg) per lactation	200-700	1690	1300	
Live weight (kg) of female	240	235	220	
Lactation Period (Days)	180	180	180	(Mohan Kharel et al., 2005)
Milk yield (kg) per lactation	720	1690	1300	
Live weight (kg) of female	225	235	230	
Lactation Period (Days)	167	120-180	120-180	(Todd M. Silk et al., 2006)
Milk yield (kg) per lactation	220	300-540	300-540	
Live weight (kg) of female	-	-	-	

1.4. Composition of Chauri Milk

Milk yield during the entire lactation period of Nak/chauri appears to be less if we compare it with the size of the animal, however, Nak/chauri milk is nutritionally rich. Nak milk contains 5.5-7.5% fat, and 4.0-5.9 % protein, the two major constituents of milk that make Nak milk thicker, denser and more viscous. Even though Nak/Chauri milk and cow milk are qualitative similar base on most of the macronutrients, the quantity of the macronutrients is higher in Nak/Chauri milk. Superiority of Nak/Chauri milk in terms of availability of macronutrients also affect its physical properties (Todd M. Silk et al., 2006; Y Ma, 2013). The following table provides composition and physical properties of Chauri breeds from China, Nepal, India and Kyrgyzstan.

Table 2. General composition and physical properties of Yak milk from different countries.

Parameters	China	Kyrgyzstan.	Nepal	India	Cow milk
Milk Solids %	16.31-19.00	17.35	17.40	17.93	13
Fat %	5.64-7.38	6.6	6.5	6.45	3.6
Protein %	4.71-6.53	6.32	5.40	5.94	3.2
Lactose %	3.49-5.31	4.62	4.60	4.68	4.7
Ash %	0.77-0.95	0.87	0.90	0.87	0.8
Specific gravity	1.03	-	-	-	1.028
Acidity %	0.2	-	-	-	0.14
Viscosity mPa.s	1.95	-	-	-	1.54

Source: (Pereira, 2014; Y Ma, 2013; Yoganandi et al., 2014)

a) Protein Composition

Based on solubility in water, milk proteins are categorized into two major types; namely casein, which is insoluble in water, and whey proteins, which are soluble in water. The casein fraction also has several types of caseins such as α-Casein, β-Casein and κ-Casein. The whey protein fraction consists of α-Lactalbumin, β-Lactoglobulin, and Serum albumin.

It is obvious from the milk composition that Nak/Chauri milk has higher concentration of total protein compared to cow milk (Table 2). Nak/Chauri milk also has higher concentration of individual caseins. For example, Nak/Chauri milk has 40.2 g/L of total caseins, which is comprised of α-Casein 16.03 g/L, β-Casein 18.2 g/L and κ-Casein 5.98 g/L (Li et al., 2010). Cow milk has lower concentration of all caseins than in Nak/Chauri milk. For example, Cow milk has 26 g/L total casein that is comprised of α-Casein 13.0 g/L, β-Casein 9.3 g/L and κ-Casein 3.3 g/L (Li et al., 2010; Pereira, 2014). Total whey proteins and individual whey proteins are also higher in Nak/Chauri milk compared to cow milk (Li et al., 2010). The proportion of β-Casein is higher (45% of total casein) in

Nak/Chauri milk, whereas, in cow milk this proportion is only 35.8%. β-Casein is believed to make soft coagulum in the stomach and is comparatively easily digested by the intestinal enzymes. For this reason, it can be inferred that Nak/Chauri milk is more easily digestible by adults and children compared to cow milk (Chen et al., 2021; Li et al., 2010). Due to higher concentration of proteins, particularly casein, yield of cheese from Nak/Chauri is 1.5-1.7 higher compared to cheese yield from cow milk (Zhang et al., 2020).

In its natural form, casein in milk remains in the form of micelles, which consist of negatively charged κ-Casein at the surface and Calcium phosphates adsorbed within the micelles. The size of the casein micelles (187.2 mm) in Nak/Chauri milk is almost twice of that compared to cow milk. The concentration of calcium is also higher in Nak/Chauri milk (2080 mg/kg) compared to 1260 mg/kg in cow milk. Due to the larger casein sizes and higher concentration of calcium, Nak/Chauri milk is coagulated faster by rennet enzyme during cheese making, and the texture of cheese curd is also firmer compared to curd made from cow milk. The firmness of curd results in harder cheese form Nak/Chauri with uniform microstructure compared to cheese made from cow milk, which is relatively softer and has irregular microstructure (Zhang et al., 2020).

b) Fat Composition

From the milk composition, it is evident that Nak/Chauri milk has almost double fat content compared to cow milk (Table 2). Milk fat is comprised of several individual fatty acids of varying molecular size and number of carbon in their molecular chains. Normally, in milk fat, fatty acids with number of carbon 4 to 20 exist in different concentration depending on various factors such as species and breed of milk animal, feed, environment, stage of lactation etc. The fatty acids with 4-8 number of carbon in the chains are called short chain fatty acids, whereas those with 10-14 carbon atoms in the chains are called medium chain fatty acids. Fatty acids with more than 14 number of carbons in the chain are categorized as long chain fatty acids. Similarly, long chain fatty acids with more than one unsaturated carbon in the chain are called poly unsaturated fatty acids or PUFA (Haug et al., 2007). Milk fats from Nak/Chauri, cow and other animal species have been further investigated for concentration of various types of fatty acids by various researchers, which

is shown in Table 3, which shows that Nak/Chauri milk is rich in total PUFA, CLA, and ω-6 fatty acids, where remaining fatty acids are comparable with cow milk.

Table 3: Comparison of major fatty acids present in Nak/Chauri milk and cow milk

Fatty acids	Fatty acids (g/100 g of fat)	
	Nak/Chauri[1]	Cow[2]
C4:0 (Butyric acid)	3.07	2.87
C6:0 (Caproic acid)	2.53	2.01
C8:0 (Caprylic acid)	1.24	1.39
C10:0 (Capric acid)	2.10	3.03
C12:0 (Lauric acid)	1.42	3.64
C14:0 (Myristic acid)	7.02	10.92
C16:0 (Palmitic acid)	26.90	28.7
C18:0 (Stearic acid)	18.26	11.23
C18:1 cis-9 (Oleic acid)	25.75	22.36
C18:2 cis-9 cis-12 (Linoleic acid)	2.69	2.57
C18:2 cis-9 trans-11 (Conjugated linoleic acid/CLA)	1.48	0.57
Total ω-3 fatty acids C18:3 (α-Linoleic acid/ALA) C20:5 (Eicosapentaenoic acid/EPA) C22:6 (Docosahexaenoic acid/DHA)	0.33	0.56
Total ω-6 fatty acids 18:2 n-6 (Linoleic acid) 18:3 n-6 (γ-Linoleic acid) 20:4 n-6 (Arachidonic acid)	4.36	2.83
Total PUFA	4.61	4.05

[1](Liu et al., 2011), fatty acid was analyzed from fat derived from milk of Nak in Tibetan plateau during August. [2](Markiewicz-Keszycka et al., 2013)

Besides fatty acids, another component of milk fat with enormous nutritional and health significance is milk fat globule membrane (MFGM). Fat is water insoluble material, which remain in milk in the form of fat globules, which are enveloped within a membrane named milk fat globule membrane. MFGM is thin but a complex membrane, which is made up of water-soluble lipids (such as phospholipids, sphingolipids), proteins including glycoproteins, and enzyme. Primarily, MFGM seems to protect the milk fat droplets and allows the fat droplets disperse in milk preventing aggregating. MFGM also has series of biologically important components that protect the new born babies against infections by enteric pathogens and boosts their immune system (Spitsberg, 2005). Generally, cow milk and Nak/Chauri milk contains comparable MFGM materials (2.1-2.8%), however, MFGM obtained from Nak/Chauri milk contains 5-10 times higher concentrations of some specific proteins such as Glycosylation-dependent cell adhesion molecule 1 (GlyCAM1), CD59 molecule and lactadherin. These proteins are believed to have antimicrobial and antitumor effects, which make MFGM from Nak/Chauri milk of higher health significance compared to that of cow milk (Ji et al., 2017).

1.5. Potential Beneficial Characteristics of Nak/Chauri Milk

Assessment of composition of Nak/Chauri milk has shown some indications about the potential superiority of Nak/Chauri milk compared to cow milk. Superiority of Nak/Chauri milk is not merely limited to higher proportions of macronutrients, but it is also due to the presence of better digestible protein fractions, presence of higher concentration of good quality fat globule membrane proteins, higher proportion of beneficial fatty acids such as PUFA, CLA, and omega-6 fatty acids, and also due to potential occurrence of aromatic and therapeutic substances derived from medicinal herbs that are present in the pastures of higher altitudes.

a) Superiority due to Better Quality Proteins

For human consumption, comparative superiority of milk obtained from certain species is based on presence or absence of certain proteins in the milk. Human milk contains higher

proportion of β-casein, but completely lacks the water soluble β-lactoglobulin. Milk from other species is thus often compared with human milk to justify the comparative suitability for human consumption. For example, camel milk is similar to human milk as it also lacks β-lactoglobulin. While comparing the other proteins, human milk contains higher proportion of β-casein, which accounts for 70% of total casein present in human milk (Meng et al., 2021). In cow milk, and Nak/Chauri milk, β-casein accounts for 35% and 45%, respectively, of the total casein present (Li et al., 2010). Based on proportion of β-casein, Nak/Chauri milk can be considered more appropriate than cow milk for human consumption. This high level of β-CN is credited to result in a smooth and soft coagulum in the human stomach, which is easily digested by the enzymes of the intestinal tract. Probably due to this reason, milk is usually given, after dilution, to babies by the Himalayan and Tibetan people to complement the breast milk (Li et al., 2010; Weiner et al., 2003). It has also been explained above that Nak/Chauri milk contains 5-10 times higher concentrations of some specific proteins such as Glycosylation-dependent cell adhesion molecule 1 (GlyCAM1), CD59 molecule and lactadherin. These proteins are believed to have antimicrobial and antitumor effects, which make MFGM from Nak/Chauri milk of higher health significance compared to that of cow milk (Ji et al., 2017).

Besides better digestibility and functionality, proteins from Nak/Chauri milk have also shown therapeutic characteristics. A study has suggested that exosomes, which are extracellular vesicles (containing proteins, lipids, nucleic acids, metabolites) generated by cells, isolated from Nak/Chauri milk can help in higher tolerance of hypoxia, i.e., low level of oxygen in our body tissues(Gao et al., 2019). This may explain the higher hypoxia tolerance of people such as Sherpa, who consume Nak/Chauri milk in their regular diet. Studies have also indicated antioxidative and anticancer effect of the peptides derived from Nak/Chauri milk. One study has demonstrated that the hydrophilic peptides derived from tryptic and chymotryptic hydrolysates of Nak milk casein showed remarkable antioxidant activities as measured by DPPH radical-scavenging assay (Kumar et al., 2013). Another group of researchers has isolated an anti-breast cancer cell peptide (TPVVVPPFL) from hydrolysate of casein obtained from Nak milk. The anticancer peptide

found to induce arresting growth of the breast cancer cell as well as inducing apoptosis or death of the cancer cells (Haofeng Gu et al., 2022).

b) Superiority due to Better Lipids

It has been demonstrated by researches that milk fat derived from Nak milk has higher concentration of PUFA, specifically the omega-6 fatty acids (Table 3), which benefits by preventing heart diseases, improving brain functions, blood clotting and muscle strength. Nak milk fat also contains almost 3 times higher concentration of the conjugated linoleic acid called CLA (Table 3). Hundreds of researches have been conducted on the potential benefits of CLA on human health; the findings of these researches have been summarized by Kim et al., (2016). CLA has been found to have anticancer activities against colon cancer, pulmonary cancer, mammary and breast cancer, prostate cancer, pancreatic cancer, and liver cancer (Arab et al., 2016; Dachev et al., 2021; Moon, 2014). CLA has also been shown to prevent the development of atherosclerosis, reduce body fat while improving lean body mass, and modulate immune and/or inflammatory responses. CLA is also useful in controlling age related health issues such as cardiovascular diseases and osteoporosis of women after menopause (Kim et al., 2016). As butter obtained from Nak/Chauri milk has almost three times higher concentration of CLA than in cow milk butter, Nak/Chauri butter has a huge potential to promote as a natural source of CLA in human diet.

c) Superiority due to Volatile and Potentially Therapeutic Compounds

The major difference between Chauri and commercial cattle farming system is that Chauri is farmed completely based on open pasture, whereas cattle are stall fed and often given concentrated feed. Due to several reason including difference in feeds, Nak/Chauri milk has characteristic stronger flavor and aroma potentially derived from pasture. The pastureland of higher altitude is generally rich in beneficial herbs and medicinal plants, which Nak/Chauri eat while grazing, and their effect definitely comes into milk. Even though there are not so many studies to demonstrate benefits of Nak/Chauri milk due to the components of pasture, some researches have established that Nak/Chauri milk has substantially higher concentration of volatile components. XiaoMei et al., (2017)

evaluated the volatile flavor components of cattle milk (CM), buffalo milk (BM) and yak milk (YM) by headspace solid-phase micro-extraction, and then analyzed by gas-chromatography mass-spectrometry (HS-SPME-GC/MS). The results showed that, 17 and 16 volatiles were identified from cow and buffalo milk, respectively. The volatiles in cow and buffalo milk were mainly ketones, acids and aldehydes. Contrary to cow and buffalo milk, 37 volatile compounds were identified from Nak milk, where additional volatiles included aromatic compounds, esters, and lactones (XiaoMei et al., 2017).

2. MATERIALS AND METHODS

2.1. Collection of Samples

Various samples of the Chauri milk and milk products were collected from different farms as shown in the Table-4.

Table 4. Various types of samples of Chauri milk and milk product collected for the study.

Samples	Sample Descriptions	Collected from
• Fresh Chauri Milk • Fresh Chauri Butter (Winter) • Sherkham Dry • Sherkham Fresh	• Morning milk of chauri was kept in ambient temperature of around 10°C for approx. 4 hours. • Milk passed boiling test before sampling. • Butter was prepared from cream separated from cold milk, by manual churning of cream and removing water. • Fresh Sherkham was prepared by	• Privately owned Chauri Farm of Ms Chhimi Sherpa • The farm was situated at upper Fera (Alt. 2700 meters) village of Solududhkunda Municipality-2, Solukhumbu

		coagulating the heated buttermilk with acidic whey and straining. • Dry Sherkham was prepared by sundrying the fresh Sherkhum into dry powder.	
Chauri (Summer)	Butter	Butter potentially from Nak, produced during Summer and kept at ambient temperature of approx. 10-15°C for approx. 6-7 months.	Privately owned shop of Jasmane Bhanjyang (Alt. 3000 meters) Solududhkunda Municipality-3, Solukhumbu.
Chauri (Summer)	Cheese	Semi-hard Chauri Cheese produced by a local processing unit of Dairy Development Corporation by standard Chauri cheese-making practices.	Hotel Sangrilla, Pikay Basecamp (Alt 3800 meters), Solukhumbu.

Figure 1. Samples packaged and delivered to the laboratory.

Samples were packed in LDPE pouches, labelled properly (Figure 1) and stored in ice-packed insulated cold box. Frozen preserved samples were transported safely to Zest Laboratory, Balkot, Bhaktapur, and stored at -18 degree until further used for analysis.

2.2. Laboratory Analyses

The samples were analysed in the Zest laboratory located in Suryabinayak Municipality-2, Balkot, Bhaktapur. The laboratory is an internationally accredited privately owned ~~reference and~~ research laboratory of Nepal.

Fresh and Dry Sherkham samples were analyzed for proximate compositions by using methods as per Association for Official Analytical Chemists (AOAC) 21st edition.

Fat was extracted from Milk, Butter and Cheese samples, and analysis of fatty acids using Gas Chromatography (GC) coupled with Flame Ionization Detector (FID) were done using Food Analysis Lab Protocol prescribed and validated by WHO (WHO, 2023). The standards of Fatty Acids were run through GC followed by sample run and the fatty acids were identified by comparing with the standards. Conjugated Linoleic Acid (CLA) was identified by comparing the chromatogram with a standard chromatogram of past research conducted using similar GC model and detector. GC protocols were repeated twice and average values were calculated.

3. RESULTS AND DISCUSSION

3.1. Composition of Sherkham

The proximate composition of Fresh and Dry Sherkham is shown in Table 5.

Table 5. Proximate composition of Fresh and Dry Sherkham made from Chauri buttermilk.

Test Parameters	Sherkham Fresh	Sherkham Dry
Moisture Content (% w/w)	67.51	12.10
Protein Content (% w/w)	25.51	61.20
Fat Content (% w/w)	1.42	15.61
Ash Content (% w/w)	0.94	2.21

Carbohydrate Content (% w/w)	3.62	8.91
Energy (Kcal/100g)	133.29	421.09

Fresh Sherkham has moisture content of 67.5% compared to 12.1% in Dry Sherkham. Protein and Fat content in Fresh Sherkham were 25.5% and 1.4%, which changed to 61.1% and 15.61%, respectively in Dry Sherkham (Table 5).

Carbohydrate content of Sherkham is very low compared to protein content, making Sherkham a high protein low carbohydrate product. As sherkham is produced by heating and coagulation of buttermilk, it can be assumed that whey protein are available in higher proportion than in products, such as paneer, made by coagulation of milk. As whey protein are considered healthier and of higher quality than rest of milk protein, Sherkham appears to be healthier than Paneer. Additionally, Sharkham is made from butter milk remained after churning of butter. Buttermilk is a very good source of milk fat globule membrane proteins (MFGM), which are considered as superfoods due to their tremendous health benefits.

3.2. Fatty acid profiles of Chauri Milk and Milk Products

Fatty acid profiles of Chauri milk and milk products are shown in Table 6. The respective chromatograms of fatty acid analysis are shown in Annex-1. Fat extracted from Chauri milk, Chauri butter of winter (freshly prepared) and summer (prepared during September-October 2022 and stored at an ambient temperature of 5-10C for 5-6 month), and Chauri cheese (prepared during summer of 2022) were analyzed for the various compositions of the fatty acids. All the samples showed fatty acids varying from C-4 to C-22. Total saturated fatty acids (SFA) were almost comparable in fat samples derived from cow milk or Chauri milk and chauri milk products. However, Chauri milk and Chauri milk products contained higher concentration of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The ratio of PUFA:SFA was higher in fats derived from Chauri milk and products compared to cow milk fat (Table 6).

Table 6. Fatty acid profiles of fats obtained from Cow milk, Chauri milk, Chauri Butter and Chauri Cheese.

Fatty Acids	Cow Milk	Chauri Milk	Chauri Butter (Winter)	*Chauri Butter (Summer)	Chauri Cheese
C4:0	3.51±0.18	4.55±0.01	3.50±0.01	4.21±0.05	4.39
C6:0	2.33±0.16	3.02±0.04	2.22±0.01	2.94±0.01	2.74
C8:0	1.35±0.13	1.53±0.01	1.08±0.01	0.93±0.60	1.38
C10:0	2.94±0.26	3.04±0.04	2.07±0.01	2.53±0.00	2.5
C12:0	3.4±0.28	2.78±0.01	1.99±0.01	2.44±0.08	2.24
**C13:0	0.11±0.01				
C14:0	11.4±1.13	9.50±0.01	7.98±0.01	9.04±0.08	8.47
C15:0	1.06±0.09	1.14±0.02	1.45±0.02	1.77±0.02	1.69
C16:0	27.45±2.84	28.67±0.05	26.33±0.00	27.86±0.25	25.99
C17:0	0.58±0.07	0.92±0.00	1.10±0.03	0.91±0.05	0.85
C18:0	13.82±1.66	15.90±0.03	18.36±0.04	18.45±0.15	17.38
C20:0	0.36±0.05	0.34±0.01	0.46±0.01	0.39±0.00	0.43
C22:0	0.21±0.02	0.15±0.01	0.16±0.01	0.18±0.01	0.17
Total SFA	68.48±6.89	71.52±0.03	66.68±0.03	71.65±0.01	68.23
(6t-14t)-C18:1	1.86±0.13	3.11±0.04	0.61±0.00	6.29±0.01	6.57
***9c-C14:1		0.35±0.01	0.32±0.01	0.53±0.01	0.46
9c-C16:1	1.55±0.16	1.87±0.03	2.04±0.01	1.67±0.02	1.48
9c-C18:1	20.37±2.64	22.32±0.06	26.55±0.03	22.28±0.24	23.03
10c-C18:1	0.29±0.07	0.38±0.01	0.54±0.01		0.77
11c-C18:1				0.28±0.00	

12c-C:18:1				0.21±0.00	
**11c-C20:1	0.11±0.02				
12c-C:22:1					
**13c-C:22:1	0.21±0.02				
Total MUFA	24.38±3.03	28.02±0.06	30.05±0.04	31.26±0.26	32.31
****C18:2 cis-9, cis-12	0.30±0.00	0.59±0.00	0.73±0.01	1.35±0.00	0.828
C18:2n-6	1.75±0.20	2.59±0.00	2.70±0.01	1.97±0.03	2.39
C18:3n-3	0.15±0.01	0.97±0.01	1.18±0.01	1.65±0.02	1.67
Total PUFA	2.20±0.20	4.14±0.02	4.60±0.00	4.96±0.05	4.88
PUFA/SFA	0.032	0.057	0.069	0.069	0.071

The values are Mean±SD, n=2.
*As per the sample provider, Summer Butter sample was mostly produced from Nak milk.
**These fatty acids were not detected in Chauri milk or Chauri Butter samples
***This fatty acid was only detected in Chauri milk or Chauri Butter samples
****This fatty acid is known as Conjugated Linoleic Acid (CLA).

Closer observation of the fatty acid profiles reveals that three fatty acids namely C13:0, 11c-C20:1 and 13c-C:22:1 were only present in fat derived from cow milk, whereas these fatty acids were completely absent in fats derived from Chauri milk and Chauri milk products (Table 6). Complete absence of these three fatty acids in Chauri milk indicates potential usage of these fatty acids as markers for adulteration of Chauri milk by cow milk. However, further detailed research is needed for confirmation. On the other hand, one fatty acid namely 9c-C14:1 was present in fats derived from Chauri milk and Chauri milk products, and completely missing in cow milk. This fatty acid may potentially be used as a marker to check adulteration of cow milk by Chauri milk.

Interestingly, two fatty acids namely 11c-C18:1 and 12c-C:18:1 were only present in the fat extracted from Chauri butter produced during summer season (Table 6). The sample of Chauri Butter prepared in summer was collected from a place called Jasmane Bhanjyan. During sample collection, the sample provider had stated that the Chauri butter from summer was prepared exclusively from Nak milk. If that statement is true, presence

of 11c-C18:1 and 12c-C:18:1 may potentially be used as a marker for Nak milk, which also needs confirmation by focused further studies.

Fats derived from Chauri milk and Chauri milk products had higher concentration of Conjugated Linoleic Acid (CLA/ C18:2 cis-9, cis-12). CLA concentration in Chauri Butter prepared during summer season had highest concentration of CLA (1.35% of total fatty acids). Higher concentration of CLA indicate that Chauri milk is healthier compared to cow milk. The highest concentration of CLA in Chauri Butter prepared during summer also indicates that Chauri milk is healthier during summer season. During summer season, Chauri graze in the pasturelands of higher altitudes, where abundant green vegetation including potentially beneficial herbs are available, which potentially lead to higher concentration of CLA in milk fat. On the other hand, Chauri are brought to the lower areas during winter season and fed with dry grasses and local feed, which might have resulted in lower quality fat with respect to CLA.

Higher concentration of CLA in fats derived from Chauri milk and Chauri milk products is consistent to the findings of past studies. CLA has been found to have anticancer activities (Arab et al., 2016; Dachev et al., 2021; Moon, 2014). CLA has also been shown to prevent the development of atherosclerosis, reduce body fat while improving lean body mass, and modulate immune and/or inflammatory responses. CLA is also useful in controlling age related health issues such as cardiovascular diseases and osteoporosis of women after menopause (Kim et al., 2016). Fat obtained from Chauri milk and milk products had almost three times higher concentration of CLA than in cow milk butter. This singular characteristic of Chauri butter adds huge value to the yak milk/butter/ghee quality to promote as a natural source of CLA in human diet.

Fats derived from Chauri milk and Chauri milk products had higher concentration of omega-3 (n-3) and Omega-6 (n-6) fatty acids. Omega 3 and 6 fatty acid benefit the consumer by preventing heart diseases, improving brain functions, blood clotting and muscle strength. In this respect, Chauri milk/butter/ghee are considered superior to cow milk counter parts.

While observing the trans-fatty acid, Chauri milk, Chauri butter of summer and cheese contained higher concentration of a trans-fatty acid, (6t-14t)-C18:1. However, Chauri butter freshly prepared in winter season had lowest level of the trans-fatty acid. Chauri butter from summer and cheese had been stored for several months before analysis that might have contributed to increased concentration of trans-fatty acid.

4. CONCLUSIONS

Sherkham made from Chauri butter milk is very rich in protein content. As buttermilk obtained from churning of cream or milk contains Milk Fat Globule Membrane proteins (MFGM), Sherkham contains higher concentration of MFGM and can be considered a healthy food, provided hygiene is maintained during each steps of Sherkham production.

Fats from Chauri milk contains higher concentration of Omega-3 and Omega-6 fatty acids, higher concentration of polyunsaturated fatty acids (PUFA), and higher concentration of conjugated linoleic acid (CLA). Chauri milk produced during summer contains these beneficial fatty acids even in higher concentration than in winter.

Few fatty acids found in cow milk fat were not found in Chauri milk fat, and few fatty acids found in Chauri milk were not found in cow milk fat. The fatty acids, which are specifically found in cow milk fat or Chauri milk fats may potentially be used as markers to determine adulteration of Chauri milk with cow milk or vice versa.

Fat obtained from Chauri butter aged for months contained higher concentration of trans fatty acids, compared to fats obtained from freshly prepared chauri butter. Trans fatty acids are considered harmful to human health. Therefore, if required for storing for long time, milk and milk products should be packaged appropriately and stored carefully to avoid increase in concentration of trans fats.

5. RECOMMENDATIONS

1. The study was conducted during winter season, when Chauri are brought to the lower lands. There is less grass available for grazing and Chauri are often fed with dry fodder. Major benefit of Chauri milk is due to the varieties of grass including herbs available in the Chauri grazing area during summer season. The study

indicated that concentration of CLA and PUFA is high in the fats extracted from Butter sample prepared from Chauri milk in summer. Therefore, it is recommended to study the fatty acid profiles of Chauri milk during summer season to confirm that Chauri milk is healthier in summer.

2. Butter obtained from Chauri milk in summer and aged till the next winter season showed higher concentration of trans fat, which is considered harmful to health. Trans fat could have been increased during improper packaging and storage of butter for extended period of time. It is recommended to study the impact of storage and packaging conditions in trans fat found in Chauri fat products. Such study will enable the producers of Chauri milk fat products (Butter and Ghee) to design appropriate packaging requirements and storage conditions.
3. The study observed some indications that few fatty acids available in Chauri milk are not available in Cow milk and vice versa. It is recommended to conduct focused and specially designed research to understand the potential of using these fatty acids as markers for adulteration of Chauri milk by cow milk.
4. Hygiene is a major aspect of Chauri milk products. The Chauri producers or handlers of Chauri milk and milk products should be trained for basic hygiene requirements at every stage of handling of Chauri milk and milk products.

5. KEY FINDING

The key finding of the study can be summarized as follows:

> ***Chauri milk fat has higher anticancer properties due to the presence of higher concentration of Conjugated Linoleic Acid (CLA). Chauri milk obtained during summer season is better compared to winter milk. Chauri milk fat also contains Omega-6 and Omega-3 fatty acids, which help preventing heart diseases, and improving brain functions, blood clotting and muscle strength.***

6. LIMITATIONS OF THE STUDY

The study has some limitations as follows:

1. Milk and milk products have been collected from some parts of Solukhumbu only.
2. Samples from summer were collected from products stored by the farmers. Some properties may differ compared to fresh products
3. This study will only provide indications of beneficial properties; nevertheless, the results may open up opportunities for more systematic researches for the interested researchers.
4. Initially, we had also planned to analyze the volatile compounds in milk and milk products. Due to the unavailability of adequately configured GC set up, we could not perform these tests.

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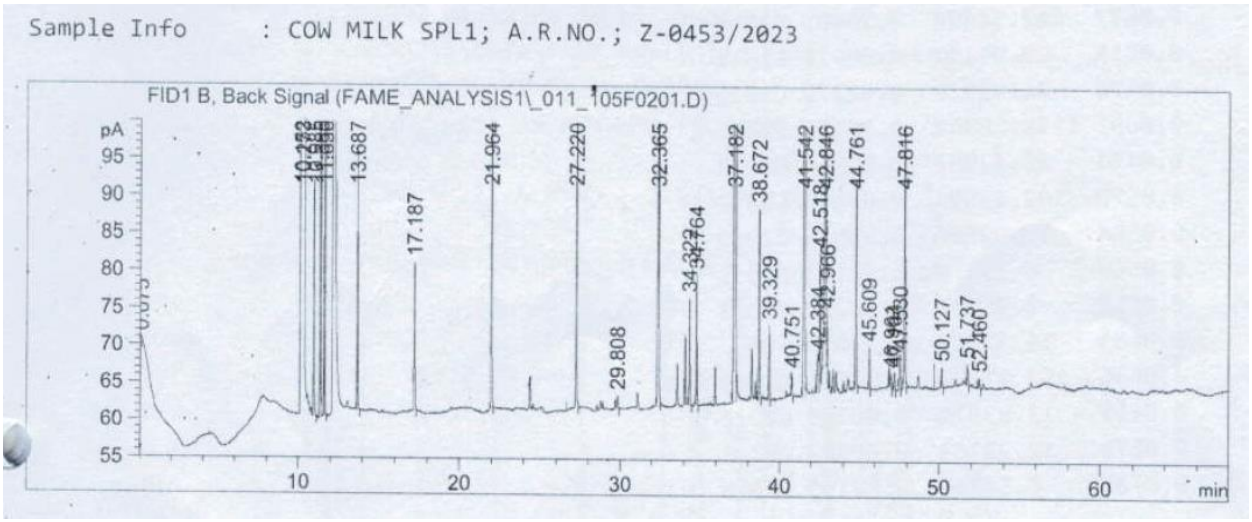
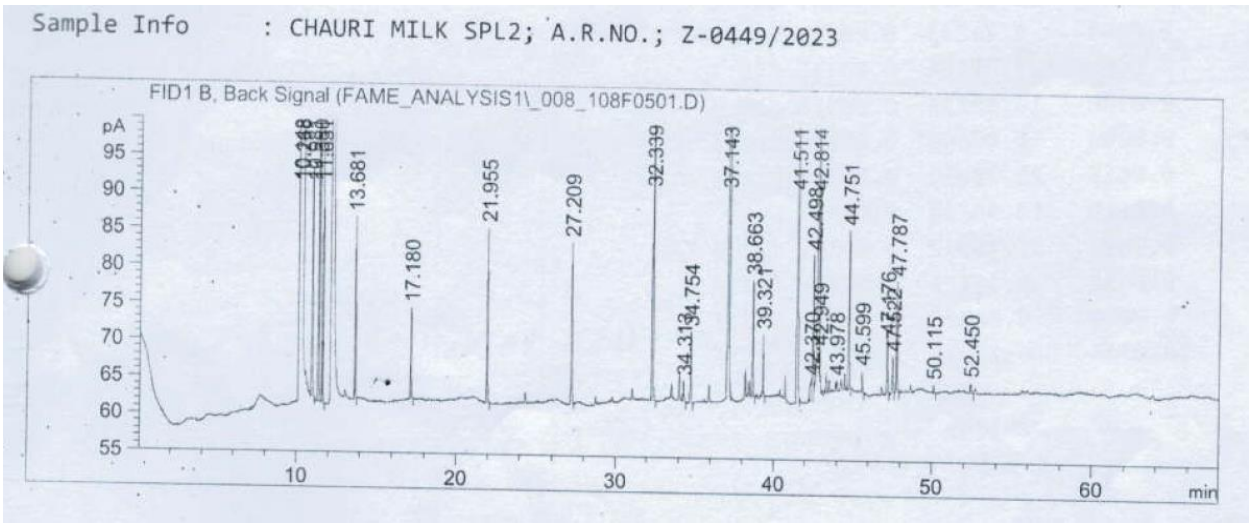
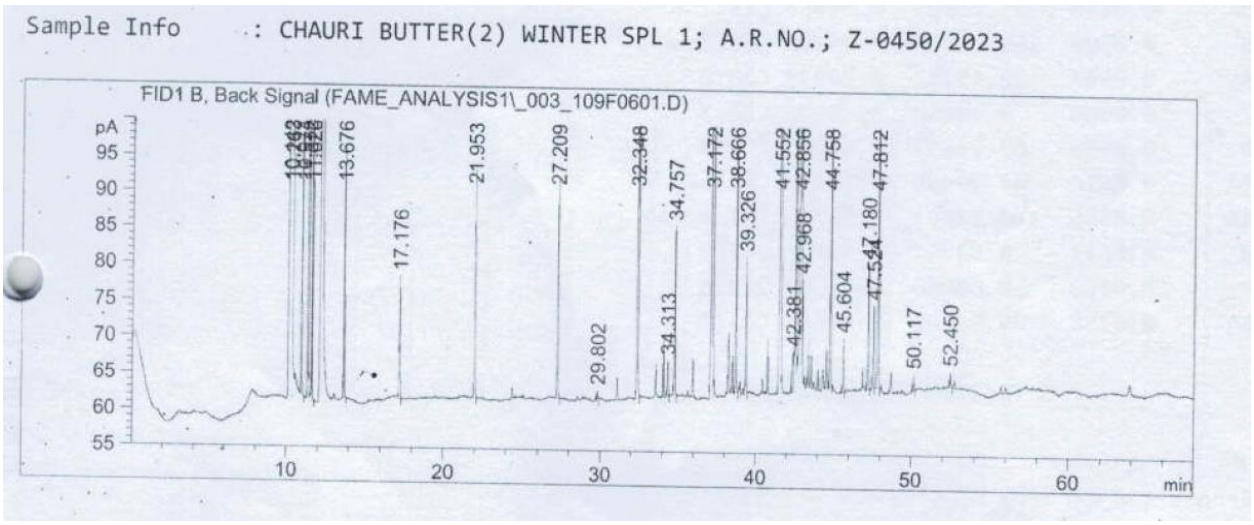
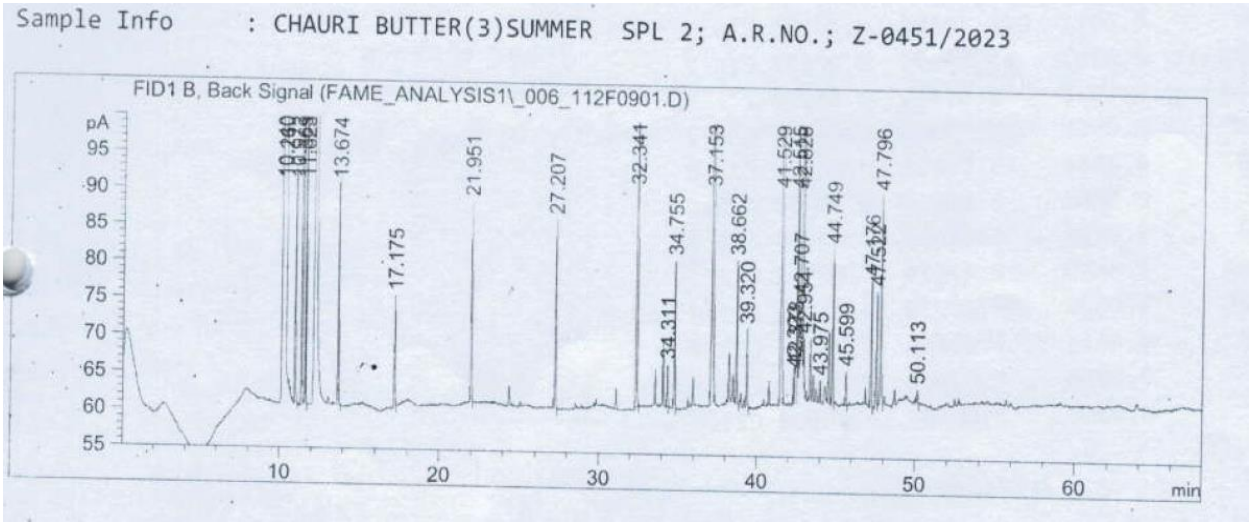
ANNEX-1.

GC Chromatogram for fatty acid determination from different milk and milk products.






Sample Info : CHAURI BUTTER(2) WINTER SPL 1; A.R.NO.; Z-0450/2023
FID1 B, Back Signal (FAME_ANALYSIS1_003_109F0601.D)
pA
95
90
85
80
75
70
65
60
55
13.676
17.176
21.953
27.209
29.802
32.348
34.313
34.757
37.172
38.666
39.326
41.552
42.381
42.856
42.968
44.758
45.604
47.180
47.812
50.117
52.450
10
20
30
40
50
60
min


Sample Info : CHAURI BUTTER(3)SUMMER SPL 2; A.R.NO.; Z-0451/2023
FID1 B, Back Signal (FAME_ANALYSIS1_006_112F0901.D)
pA
95
90
85
80
75
70
65
60
55
13.674
17.175
21.951
27.207
32.341
34.311
34.755
37.153
38.662
39.320
41.529
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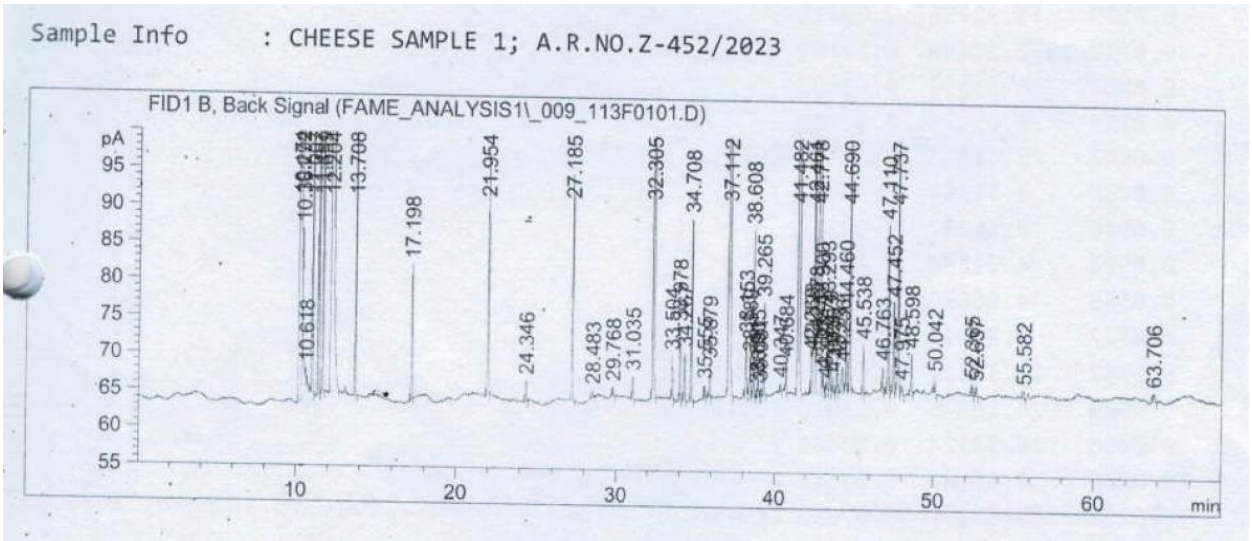
47.796
50.113
10
20
30
40
50
60
min


Sample Info : CHEESE SAMPLE 1; A.R.NO.Z-452/2023
FID1 B, Back Signal (FAME_ANALYSIS1_009_113F0101.D)
pA
95
90
85
80
75
70
65
60
55
13.700
17.198
21.954
24.346
27.185
28.483
29.768
31.035
32.305
34.708
37.112
38.608
39.265
41.482
44.460
44.690
45.538
46.763
47.452
47.737
50.042
55.582
63.706
10
20
30
40
50
60
min