CHAPTER – 5

RESULTS AND DISCUSSION

The results of the present study entitled "Probiotic profiling and organoleptic evaluation of traditional cereal based fermented drink and its market potential" are presented, discussed and interpreted in this chapter. These results are presented under four main phases according to the objectives of the study.

Phase I - Acceptability of FOS added *ambils* in terms of various organoleptic attributes and overall acceptability using composite score.

Phase II - Probiotic profiling of buttermilk and modified *ambils*

Phase III - Physicochemical properties, nutritional composition of buttermilk and ambils and shelf life of fresh and packaged *ambils*.

Phase IV - Consumer acceptability of packaged ambil and its market potential.

Phase I - Acceptability of FOS added *ambils* in terms of various organoleptic attributes and overall acceptability.

Ambil is an indigenous natural cereal based lactic fermented beverage popular in North-Western India, Maharashtra ("*Rabadi", "Ambil*") and Southern India ("*Ambli*"). So far, the technology of *Ambil* preparation has remained a household art, which results in wide variation and in terms of sensory qualities and shelf stability. (Modha and Pal, 2011). Greater prospects exists in India for value-addition and improving health benefits of milk and milk by-products by combining with traditional cereals and applying advanced technologies for their processing and preservation. No work of technological or scientific significance has been reported in the literature on either standardization of method or large-scale production of *Ambil* with standard composition. Therefore, this phase of research work was undertaken to study the acceptability trials of Fructooligosaccharide (FOS) added various cereal-millet based *ambils* viz. rice, pearl millet, barley and finger millet using different colors and flavours.

The results of this phase are divided into following sections:

5.0.1 Assessment of organoleptic properties of FOS added rice *ambil* with different favours

5.0.2 Assessment of organoleptic properties of FOS added barley *ambil* with different favours

5.0.3 Assessment of organoleptic properties of FOS added pearl millet *ambil* with different favours

5.0.4 Assessment of organoleptic properties of FOS added finger millet *ambil* with different favours

5.0.5 Assessment of organoleptic properties of salt-cumin flavoured *ambils* with different cereals

5.0.1 Assessment of organoleptic properties in FOS added rice *ambil* with different flavours

The organoleptic scores of rice *ambil* with different flavours varied significantly (Table 5.1)

Color and appearance attributes of the rice *ambils* varied significantly with different flavours and colors. Rose color was the most acceptable, followed by khus (9.19) and salt-cumin (9.04). The color of chocolate (8.89) flavoured *ambil* had the least acceptability. Non-significant difference in the consistency scores was reported in the different flavours of rice *ambils*. The acceptability in terms of taste and flavour varied significantly ranging from 25.64 to 27.47. Maximum score was obtained by salt-cumin (27.47) flavoured rice *ambil*, followed by rose (26.92), khus (25.71) and chocolate (25.64).

Mouthfeel of the *ambils* also showed significant difference. Maximum score was obtained by salt-cumin flavoured (8.97) *ambil*. Slight reduction in the scores was observed from rose (8.74), chocolate (8.62), and khus (8.42) flavoured ambils. The scores for after taste ranged from 8.32 to 8.88, with a significant difference amongst all the flavours. In spite of this difference, all products were in acceptable range. Thus it can be concluded that none of these drinks had shown any unacceptable after taste. Overall acceptability showed a significant difference ($p<0.000$) with salt-cumin (18.17) flavour having the highest acceptability, preceded by chocolate (17.11) and rose (17.69) flavoured ambils. Khus flavour scored the minimum with 16.69, depicting that it was least liked by the panel members.

Table 5.1 Comparison for organoleptic properties of different flavours of rice ambils

Parameters	Rose	Khus	Chocolate	Salt-cumin	F value	p - value
	Mean ±SD	**Mean ±SD**	**Mean ±SD**	**Mean ±SD**		
Color (10)	9.40 [a] ± 0.83	9.19 [ab] ± 1.13	8.89 [b] ± 1.17	9.04 [b] ± 0.97	3.23*	0.023
Consistency (10)	9.06 ± 0.90	9.00 ± 1.02	9.18 ± 0.82	9.18 ± 0.82	0.74[NS]	0.529
Taste and flavour (30)	26.92 [a] ± 3.06	25.71 [b] ± 3.84	25.64 [b] ± 3.78	27.47 [a] ± 2.99	4.99**	0.002
Mouthfeel (10)	8.74 [a] ± 1.17	8.42 [a] ± 1.40	8.62 [a] ± 1.18	8.97 [ab] ± 0.94	2.73*	0.044
After taste (10)	8.83 [a] ± 1.33	8.40 [ab] ± 1.37	8.32 [b] ± 1.46	8.88 [c] ± 1.10	3.39*	0.018
Absence of defects (10)	8.71 ± 1.32	8.65 ± 1.22	8.72 ± 1.24	8.89 ± 1.06	0.50[NS]	0.682
Overall acceptability (20)	17.69 [acd] ± 2.34	16.69 [be] ± 3.04	17.11 [ce] ± 2.08	18.17 [d] ± 2.20	5.02**	0.002

- Mean values represent the average of 25 determinants in triplicate
- a, b, c – The non-identical letters in any 2 columns within the rows denote a significant difference at a minimum of 5% level
- NS – the difference between the mean values within the columns is not significant.
- Level of significance in increasing order – (*p<0.05, **p<0.01, ***p<0.001)

5.0.2 Assessment of organoleptic properties in FOS added barley *ambil* with different flavours

The organoleptic scores of barley *ambil* with different flavours varied significantly (Table 5.2). Considering the color and appearance of all the *ambils*, no significant difference was found between colors, indicating that all were equally acceptable by the panel. The scores ranged from 8.64 for chocolate colored ambil followed by salt-cumin (8.75), khus (8.90) and rose (9.00) colored ambils. The choice of taste and flavour varied significantly. Maximum acceptability was found for salt-cumin (25.64) flavoured barley *ambil*, followed by rose (24.32) and chocolate (24.07) and least by khus (23.75) flavoured ambil.

Mouthfeel of the product were also significantly different. Salt-cumin (8.47) flavoured ambil had the highest score for good mouth feel, followed by chocolate (7.99), rose (7.79) and khus (7.67) flavoured ambil. Significant difference in the after taste of the different flavoured ambils was observed. Maximum scores obtained by salt-cumin (8.60), depicting that salt cumin is the most acceptable flavour of ambils and had shown minimum after taste. Chocolate (8.12), rose (8.06) and khus (8.01) flavoured ambils were also acceptable and had shown a slight after taste as compared to salt-cumin flavoured ambil.

Overall acceptability showed a significant difference with salt-cumin (17.51) flavoured ambil having the highest acceptability, followed by chocolate (16.18), rose (16.03) and khus (15.86).

Table 5.2. Comparison for organoleptic properties of different flavours of barley ambils

Parameters	Rose	Khus	Chocolate	Salt-cumin	F value	p - value
	Mean ±SD	**Mean ±SD**	**Mean ±SD**	**Mean ±SD**		
Color (10)	9.00 ± 1.00	8.90 ± 0.99	8.64 ± 1.24	8.75 ± 1.05	1.57^{NS}	0.195
Consistency (10)	8.50 ± 1.00	8.49 ± 0.97	8.62 ± 0.98	8.83 ± 0.91	1.96 ^{NS}	0.119
Taste and flavour (30)	24.32 ^{ab} ± 4.44	23.75 ^{a} ± 4.49	24.07 ^{a} ± 4.01	25.64 ^{b} ± 3.88	2.78*	0.041
Mouthfeel (10)	7.79 ^{a} ± 1.38	7.67 ^{a} ± 1.44	7.99 ^{a} ± 1.44	8.47 ^{b} ± 1.13	4.88**	0.003
After taste (10)	8.06 ^{a} ± 1.38	8.01 ^{a} ± 1.21	8.12 ^{a} ± 1.34	8.60 ^{b} ± 1.19	3.17*	0.025
Absence of defects (10)	8.14 ± 1.27	8.19 ± 1.27	8.24 ± 1.25	8.60 ± 1.17	2.01 ^{NS}	0.110
Overall acceptability (20)	16.03 ^{a} ± 2.42	15.86 ^{a} ± 2.30	16.18 ^{a} ± 2.32	17.51 ^{b} ± 2.08	7.87***	0.000

- Mean values represent the average of 25 determinants in triplicate
- a, b, c – The non-identical letters in any 2 columns within the rows denote a significant difference at a minimum of 5% level
- NS – the difference between the mean values within the columns is not significant.
- Level of significance in increasing order – (*p<0.05, **p<0.01, ***p<0.001)

5.0.3 Assessment of organoleptic properties in FOS added pearl millet *ambil* with different flavours

The organoleptic scores of pearl millet *ambil* with different flavours varied significantly (Table 5.3)

The color and appearance of the pearl millet *ambils*, varied significantly with the colors used, ranging from 8.49 (chocolate color) to 9.04 (rose color). Pink color of rose *ambil* was more appealing to the panel members as compared to others, thus confirming the best acceptability. A non-significant difference in the consistency scores was reported in the different flavours of pearl millet *ambils*. The choice of taste and flavour depicted significant difference (p<0.001). Maximum acceptability was found for salt-cumin (26.07) flavoured pearl millet *ambil*, followed by rose (25.36), khus (24.1) and chocolate (24.08).

Significant difference in the mouthfeel of the pearl millet ambils with different flavours was observed. Salt-cumin (8.38) had the highest score for good mouth feel, followed by rose (8.25) and khus (7.92). Least was scored by chocolate flavoured *ambil* (7.90). The after taste of the different flavoured ambils was observed in the range of 7.76 to 8.38 and were significantly different from each other. Maximum score was obtained by salt-cumin (8.38) flavoured ambil followed by rose (8.08), chocolate (7.81) and khus (7.76). None of the flavours of pearl millet *ambil* had shown any defects and were all acceptable by the panel members.

Overall acceptability showed a significant difference ($p<0.000$) with salt-cumin flavoured ambil (17.49) having the highest acceptability followed by rose (16.89) and khus (16.0). Least was scored by chocolate flavoured *ambil* (15.83).

Table 5.3. Comparison for organoleptic properties of different flavours of pearl millet ambils

Parameters	Rose	Khus	Chocolate	Salt-cumin	F value	p - value
	Mean ±SD	**Mean ±SD**	**Mean ±SD**	**Mean ±SD**		
Color (10)	9.04 ± 0.91 ^{a}	8.97 ± 1.10 ^{a}	8.49 ± 1.30 ^{b}	8.74 ± 1.19 ^{ab}	3.53*	0.015
Consistency (10)	8.71 ± 1.04	8.71 ± 1.08	8.60 ± 1.07	8.86 ± 1.16	0.71^{NS}	0.546
Taste and flavour (30)	25.36 ± 3.51 ^{ab}	24.1 ± 4.37 ^{a}	24.08 ± 4.27 ^{a}	26.07 ± 3.90 ^{b}	4.27**	0.006
Mouthfeel (10)	8.25 ± 1.08 ^{ab}	7.92 ± 1.21 ^{a}	7.90 ± 1.29 ^{a}	8.38 ± 1.34 ^{b}	2.65*	0.049
After taste (10)	8.08 ± 1.25 ^{ab}	7.76 ± 1.28 ^{a}	7.81 ± 1.31 ^{a}	8.38 ± 1.33 ^{b}	3.431*	0.018
Absence of defects (10)	8.47 ± 1.22	8.24 ± 1.37	8.40 ± 1.20	8.54 ± 1.29	0.756^{NS}	0.520
Overall acceptability (20)	16.89 ± 1.95 ^{a}	16.00 ± 2.35 ^{bc}	15.83 ± 2.57 ^{c}	17.49 ± 2.37 ^{c}	8.02***	0.000

- Mean values represent the average of 25 determinants in triplicate
- a, b, c – The non-identical letters in any 2 columns within the rows denote a significant difference at a minimum of 5% level
- NS – the difference between the mean values within the columns is not significant.
- Level of significance in increasing order – (*p<0.05, **p<0.01, ***p<0.001)

5.0.4 Assessment of organoleptic properties in FOS added finger millet *ambil* with different flavours

The organoleptic scores of finger millet *ambil* with different flavours varied significantly (Table 5.4)

Rose and salt-cumin flavoured ambils both were equally (8.38) liked by the panel members. Least acceptability was for Khus (8.18) flavoured *ambil*. The acceptability of taste and flavour varied significantly ranging from 23.22 to 25.15. Maximum was found for salt-cumin (25.15) flavoured finger millet *ambil*, followed by rose (23.69), chocolate (23.24) and khus (23.22). Mouthfeel of the *ambils* were also significantly different. Maximum score was obtained by salt-cumin (8.17) flavoured *ambil*. Slight reduction in the scores was reported from chocolate (7.71), rose (7.56) and khus (7.49) flavoured ambils. None of the flavours of ambils had shown any significant after taste. The scores ranged from 7.78 to 8.31, with no significant difference amongst all the flavours of ambils. Overall acceptability showed a significant difference (p<0.000) with salt-cumin (17.19) flavoured ambil having the highest acceptability, preceded by chocolate (15.99) and rose (15.82).

Table 5.4. Comparison for organoleptic properties of different flavours of finger millet ambils

Parameters	Rose	Khus	Chocolate	Salt-cumin	F value	p - value
	Mean ±SD	**Mean ±SD**	**Mean ±SD**	**Mean ±SD**		
Color (10)	8.38 ± 1.22	8.21 ± 1.17	8.18 ± 1.46	8.38 ± 1.08	0.511 NS	0.675
Consistency (10)	8.24 ± 1.25	8.21 ± 1.17	8.08 ± 1.47	8.35 ± 1.30	0.498 NS	0.684
Taste and flavour (30)	23.69 [a] ± 4.14	23.24 [a] ± 4.43	23.22 [a] ± 4.80	25.15 [b] ± 3.88	3.18*	0.024
Mouthfeel (10)	7.56 [a] ± 1.33	7.49 [a] ± 1.42	7.71 [a] ± 1.52	8.17 [b] ± 1.30	3.45*	0.017
After taste (10)	7.93 ± 1.24	7.78 ± 1.65	8.01 ± 1.40	8.31 ± 1.25	2.19 NS	0.089
Absence of defects (10)	8.14 ± 1.39	7.97 ± 1.40	8.00 ± 1.58	8.24 ± 1.39	0.523 NS	0.667
Overall acceptability (20)	15.82 [a] ± 2.34	15.60 [a] ± 2.36	15.99 [a] ± 2.58	17.19 [b] ± 2.62	6.43***	0.000

- Mean values represent the average of 25 determinants in triplicate
- a, b, c – The non-identical letters in any 2 columns within the rows denote a significant difference at a minimum of 5% level
- NS – the difference between the mean values within the columns is not significant.
- Level of significance in increasing order – (*p<0.05, **p<0.01, ***p<0.001)

5.0.5 Comparison for organoleptic properties of different cereal-millet based ambils with salt cumin flavor

The organoleptic evaluation of all the cereal -millet based *ambils* on the basis of salt-cumin flavour revealed that all the *ambils* had shown significant difference (p<0.000) in terms of all the organoleptic properties (Table 5.5). The consistency, taste and mouthfeel of the *ambils* varied significantly. Maximum acceptability was seen for rice *ambil* (18.17), followed by barley (17.51), barley (17.49) and finger millet (17.19) *ambil*.

Table 5.5 Comparison for organoleptic properties of different cereal-millet based ambils with salt cumin flavor

Parameters	Rice	Barley	Pearl millet	Finger millet	F value	p - value
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD		
Color (10)	9.04 [b] ± 0.97	8.75 ± 1.05	8.74 ± 1.19 [ab]	8.38 ± 1.08	4.56**	0.004
Consistency (10)	9.18 ± 0.82	8.83 ± 0.91	8.86 ± 1.16	8.35 ± 1.30	7.42***	0.000
Taste and flavour (30)	27.47 [a] ± 2.99	25.64 [b] ± 3.88	26.07 ± 3.90 [b]	25.15 [b] ± 3.88	5.27***	0.001
Mouthfeel (10)	8.97 [ab] ± 0.94	8.47 [b] ± 1.13	8.38 ± 1.34 [b]	8.17 [b] ± 1.30	5.90***	0.001
After taste (10)	8.88 [c] ± 1.10	8.60 [b] ± 1.19	8.38 ± 1.33 [b]	8.31 ± 1.25	3.16***	0.025
Absence of defects (10)	8.89 ± 1.06	8.60 ± 1.17	8.54 ± 1.29	8.24 ± 1.39	2.94*	0.033
Overall acceptability (20)	18.17 [d] ± 2.20	17.51 [b] ± 2.08	17.49 ± 2.37 [c]	17.19 [b] ± 2.62	2.42 [NS]	0.066

- Mean values represent the average of 25 determinants in triplicate
- a, b, c – The non-identical letters in any 2 columns within the rows denote a significant difference at a minimum of 5% level
- NS – the difference between the mean values within the columns is not significant.
- Level of significance in increasing order – (*p<0.05, **p<0.01, ***p<0.001)

Amongst all the ambils rice ambil had the maximum acceptability and was scored the highest for salt cumin flavour, followed by barley ambil, pearl millet ambil and finger millet ambil respectively. As can be seen in Table 5.5, salt cumin flavour was considered best for all the cereal-millet based ambils.

Result highlights of Phase I

- *Amongst all the cereal-millet based ambils, rice ambil was most acceptable by panel members.*
- *Salt-cumin flavour had the highest acceptability amongst all the cereal-millet based ambils including rice ambil (18.17), barley ambil (17.51), pearl millet ambil (17.49) and finger millet ambil (17.19).*
- *Among all the ambils, the consistency score reduced for barley, pearl millet and finger millet ambils as compared to rice ambil.*
- *All the sensory attributes decreased for pearl millet and finger millet ambils as compared to rice and barley ambils.*
- *Color, consistency, taste and overall acceptability of finger millet ambils was least acceptable amongst all the ambils*
- *Rose color scored significantly ($p<0.01$) higher in rice and pearl millet ambils.*
- *Salt cumin flavour could mask the smaller changes seen in organoleptic properties of all the cereal ambils prepared with different flavours.*
- *All the ambils prepared from different cereals and flavours had an overall acceptability score of more than 75%.*
- *The overall acceptability scores for rice ambil was highest amongst all and scored 88% scores.*
- *Addition of 5% FOS as a prebiotic to ambils did not alter the taste of all the ambils with different flavours.*

Discussion

Today's lifestyle has led to vital changes in food pattern. People are more concerned about health and there is an increasing demand for foods which are beneficial to their health. Various FOS based products like beverage concentrates, spreads and honey have been studied successfully and their processes have been patented. (Ramesh *et al*, 2004; Renuka *et al*, 2009).

Functional foods and beverages must show, at least, similar performance in any sensory test as conventional foods. In the present study a traditional beverage *ambil* was modified using different cereals with flavours and addition of FOS, and had shown good acceptability amongst panel members. Studies on developing *ambil* using sorghum and the effect of locally available methods of preparation on the organoleptic properties have also shown good acceptability (Surpam *et al*, 2014). Similar study was conducted to develop an oat based drink called as "Oat drink" using oats as a substrate and was fermented at 37 °C for 6–10 hrs showed a good acceptability amongst panel members (Angelov *et al*, 2006).

Research findings have revealed that substitution of some commonly consumed food products like cakes, soups, *ambli*, malt and muffins with finger millet and found a good score for appearance, texture and flavour with overall acceptability scores. (Begum *et al*, 2017; Yanagi *et al*, 2013). A study conducted on FOS added soup and beverages namely, butter milk, lemon juice, milk and tomato soup at 2.5 per cent, 4 per cent, 5 per cent, 6 per cent, 7.5 per cent has shown positive results on the overall acceptability of the products (Gupta and Sheth, 2011).

Organoleptic assessment of various *ambils* developed and studied in the present study revealed a significant difference in the acceptability of these *ambils* with regards to colors, taste, flavours, mouthfeel, after taste, defects and overall acceptability.

In foods and beverages, the consumer often assesses the initial quality of the product by its color and appearance. The appearance and color of these products are thus the primary indicators of perceived quality. The color of the developed *ambils* were chosen according to the flavours, *viz*. pink for rose, green for khus, brown for chocolate and white for salt-cumin flavoured *ambils*. In terms of color, pink color of rose was liked most by the panellists for all the cereal-millet based *ambils*, followed by khus, chocolate and white color of salt-cumin. Patil, 2012 have stated that color and appearance are the major factors affecting consumer choices for buying a food product as it affects the adrenal glands, tempting the taste buds. It was also documented that pink is perceived as sweet and more appealing. Lee, 2002 states that colors improve efficiency of the message and make them appear real as they form a visual concord of the elements projected in the ad. They can also lead to primary source of subconscious persuasion, but wrong colors may keep the customers away from considering product for purchasing.

Consistency is another important parameter to study the likability of a beverage. In the present study, consistency of all the flavours of rice *ambils* were more acceptable to the panel members as compared to the flavours of other cereal-millet based *ambils*. It can be due to more coarse texture of the flour of pearl millet, finger millet and barley as compared to rice, thus affecting the consistency of the developed *ambils*. Also, rice starch granules are the smallest known to exist in cereal grains, with the size reported in the range of 2 to 7 μm (Vandeputte and Delcour 2004). These granules have smooth surface thus providing a smooth consistency to the *ambil* as compared to other cereals. Similar results have also been reported by Hasan *et al* 2012, showing higher acceptability of rice based probiotic beverage in comparison to millets.

Taste and flavour are considered to be the prime factors for any organoleptic studies. The taste and flavour of rice *ambils* was preferred over all the other *ambils* of different flavours. Rice is a staple food and is consumed by majority of population in India (Ashkani *et al*, 2015) as compared to other millets or barley and people are more used to the taste of rice, this may be the reason of more acceptability of rice *ambils*. Similar results on the more preference and consumption of rice over barley and other cereals have also been reported (Khatkar and Dangi, 2016; Nagaraj *et al*, 2012). A study conducted by Jadoun *et al*, 2011, acceptability score of Bajra Lassi was evaluated by 120 consumers by using 9 point hedonic scale. The mean sensory score for the taste was found to be 6.88 and 7.57. It indicated that although a traditional composite dairy food, the findings reflect the popularity and willingness of urban consumers for traditional foods in modern presentation.

A study was undertaken with a view to analyse and compare the sensory quality and nutritive value of food products from millet with rice. The formulated products viz. *laddu, halwa* and *biryani* from millets and rice (control) were analysed for their sensory qualities. Among the products prepared, there was non significant difference with regard to the colour, flavour, texture, appearance and overall acceptability of millet *laddu* and *halwa* when compared to control (Verma *et al*, 2015). Similar study was conducted and finger millet lassi was developed and studied for its sensory acceptability, which showed that the overall acceptability of finger millet lassi increases as increase in the level of finger millet flour (Pardhi *et al*, 2014). The results of the above documented studies were contradictory to the results obtained by the present study, that is ambils prepared using rice were highly acceptable as compared to finger millet ambil and had shown a significant difference.

Amongst all the flavours of ambils, salt-cumin flavoured rice *ambil* was most liked by the panel, and chocolate the least. Similar results were also reported by Modi, 2015 where he studied the most acceptable flavours of ice cream amongst the consumers, and documented that chocolate flavour of ice cream was least acceptable by the adults. In a study conducted by Mali and Inamdar, 2010 consumer preference for various ice cream flavours of Amul was studied. The results documented that out of the most popular flavours vanilla, butterscotch, chocolate and strawberry, chocolate was least preferred by adults (16%) and most preferred by the age group of 11-15 years, which is contradictory to the present study.

Mouthfeel, after taste and absence of defects also play a significant role in the acceptability of any food product, as they are directly related to the first bite or sip of any food. Rice has shown the best acceptability in terms of these three parameters, followed by and pearl millet and barley due to their mild taste. In the present study it was observed that the overall acceptability of rice *ambil* was better as compared to all the other *ambils*, which can be assumed because of the better mouthfeel and taste of rice. Similar results have also been reported by Durojaiye *et al* (2010) during the development of an African traditional drink *fura*.

The overall acceptability of the *ambils* developed in this study with respect to the addition of FOS, even though it imparts a sweet taste, has not affected the overall taste, aftertaste and mouthfeel of the product. Similar results have been documented by Parnami *et al*, where cookies and bread that were fortified with prebiotic inulin were highly acceptable (Parnami and Sheth, 2010)

Acceptability of FOS have been seen in commonly consumed Indian food products like *muthiya*, vegetable *cheela* (Assudani *et al*, 2014), *chapatti*, *dhokla* (Mahendra and Sheth, 2013), *kachori* and *paratha* (Jain *et al*, 2014), and all have shown good acceptability.

Fructooligosaccharide is indeed present in commonly consumed plants, it can be added to normal food products, as it modulates key physiological functions including lipid metabolism, it modulates the composition of gut microflora, which plays a major role in gastrointestinal physiology.

Several food scientists have, however, endeavoured to develop non-dairy, cereal based probiotic and/or synbiotic products (Enujiugha and Badejo, 2017; Ranadheera and Vidanarachchi, 2017; Markowiak and Slizewska, 2017). The fermented beverage developed in this study had showed good acceptability amongst the panel members, this can be subjected due to lactic acid bacteria and yeasts that are the predominant microorganisms during the cereals fermentation. The activities of lactic acid bacteria produce many antimicrobials and inhibitory substances during fermentation while the yeast contributes to flavour enhancement (Kumari *et al*, 2015).

Cereals and millets have been an essential part of the human diet since the beginning of agriculture and are most economic source of energy (Das *et al*, 2012). These are nutritionally rich and occupy an important place in the diet of people in many regions of the world. Rice and barley are commonly consumed cereals in Indian households. Although millets are nutritionally superior to cereals their utilization as food is mostly confined to the traditional consumers and population of lower economic strata (Yanagi *et al*, 2013). The formulation of *ambils* in the present research study led to development of healthy beverages using locally available ingredients and addition of FOS further enhance the quality.

Further research can be undertaken by incorporating indigenous pulses, other cereals and flavours to provide a wider variety, enhancing the nutritional value and further study its organoleptic qualities and shelf stability.

Conclusion

- *All the cereal-millet based ambils with different flavors have shown good acceptability in terms of various organoleptic attributes and salt cumin ambil was the most preferred followed by rose flavor.*
- *Amongst the various FOS added cereal and millet based ambils, rice and barley ambils were the most preferred ones and there was no significant difference in the OA scores of all the ambils.*
- *Indigenous pulses, other cereals and flavours can be used to provide a wider variety, enhancing the nutritional value and further study its organoleptic qualities and shelf stability.*

Phase II - Probiotic profiling of modified *ambils*

Over the last decade, demand for "healthy" foods and beverages has increased in many parts of the world (Ozen *et al* 2012). Functional food generally contain health-promoting components beyond traditional nutrients. It can be an unmodified natural food; a food in which a component has been enhanced through special growing conditions, breeding, or biotechnological means or a food to which a component has been added to provide benefits (Pravst 2012). One way of creating a functional food is by inclusion of ingredients such as probiotics and prebiotics to levels that enable the consumer to derive optimal health benefits.

Within dairy beverages, fresh milk, fermented milk, and yogurt drinks are the most common products, as they are considered excellent vehicles for probiotics (Reid, 2015).

Lactobacillus and Bifidobacterium are the most commercially found species in the probiotic products (Krastanov and Denkova, 2012). Among these *Lactobacillus rhamnosus, Lactobacillus acidophilus, Bifidobacterium longum*, and *Bifidobacterium bifidum* are mainly present in functional foods. Recently, various genotype-based methods have been adopted as useful ways for identification of bacteria (Tshikhudo *et al*, 2013). Single PCR, using primers that target variable regions in universal genes, such as the 16S rRNA, 16S-23S rRNA intergenic spacer region (ISR), or 23S rRNA, have successfully detected and identified the LAB (Sabat *et al*, 2017; Stamatoski *et al*, 2016; Adzitey *et al*, 2013).

In the present study, a dairy based health drink was developed using functional ingredient FOS and commonly consumed cereals rice, barley, pearl millet and finger millet with buttermilk followed by fermentation to enhance the growth of beneficial bacterial species. The major objective of this phase of the study was to isolate Lactobacillus and Bifidobacterium from the developed functional beverage and to identify the isolates at the strain level.

For achieving the desired objective, the *ambils* were prepared in the Genelon Institute of Life Sciences, Bangalore where they were studied for their probiotic content.

The results of this phase is divided into the following sections:

5.1.1 Morphological characteristics of the bacterial colonies

5.1.2 Bifidobacterium and Lactobacillus species isolated from buttermilk and ambils

5.1.3 Isolation of Bifidobacterium strains from buttermilk

5.1.4 Isolation of Lactobacillus strains from buttermilk

5.1.5 Isolation of Bifidobacterium strains from rice *ambil*

5.1.6 Isolation of Lactobacillus strains from rice *ambil*

5.1.7 Isolation of Bifidobacterium strains from pearl millet *ambil*

5.1.8 Isolation of Lactobacillus strains from pearl millet *ambil*

5.1.9 Isolation of Bifidobacterium strains from barley *ambil*

5.1.10 Isolation of Lactobacillus strains from barley *ambil*

5.1.11 Isolation of Bifidobacterium strains from finger millet *ambil*

5.1.12 Isolation of Lactobacillus strains from finger millet *ambil*

5.1.1 Morphological characteristics of the bacterial colonies

The isolates from *ambils* were studied for their morphological characteristics viz., type of colony, color, margin, elevation and opacity. Isolated colonies appeared from small to large in their shape. The colour of colonies ranged from off white to shiny white and creamy white. Margins were entire in all the isolates, except a few irregular ones. Elevations of the isolated colonies were flat, raised and convex. The opacity of the isolates was translucent and opaque in nature and color pigments were absent in all pure colonies of the isolates.

5.1.2 Bifidobacterium and Lactobacillus species isolated from buttermilk and ambils

A summary of all the Lactobacillus and Bifidobacterium strains isolated from the buttermilk and all ambils have been documented in Table 5.6

Table 5.6 Bifidobacterium and Lactobacillus isolated from buttermilk and *ambils*

Bacterial species	Buttermilk	Rice *ambil*	Barley *ambil*	Pearl millet *ambil*	Finger millet *ambil*
Bifidobacterium bifidum	-	-	-	+	-
Bifidobacterium thermacidophilum	-	-	-	+	-
Bifidobacterium thermophilium	-	-	-	+	-
Bifidobacterium animalis	-	-	-	-	-
Bifidobacterium stercoris	+	+	+	-	-
Bifidobacterium faecale	-	+	+	-	-
Bifidobacterium adolescentis	-	+	+	-	-
Bifidobacterium ruminantium	-	+	+	-	-
Bifidobacterium choerinum	-	-	-	-	+
Bifidobacterium gallicum	-	-	-	-	+
Bifidobacterium catenulatum	+	+	+	-	-
Bifidobacterium pseudocatenulatum	+	+	+	+	-
Bifidobacterium kashiwanohense	+	+	+	-	-
Bifidobacterium angulatum	+	+	+	-	-
Bifidobacterium lemurum	-	-	-	+	-
Bifidobacterium pseudolongum	-	-	-	-	+
Bifidobacterium cuniculi	-	-	-	-	+
Bifidobacterium breve	-	-	-	-	+
Bifidobacterium scardovii	-	-	-	+	-
Bifidobacterium merycicum	+	-	-	-	-
Bifidobacterium callitrichos	+	-	-	-	-
Lactobacillus acidophilus	+	+	+	+	+
Lactobacillus reuteri	+	-	+	+	-
Lactobacillus casei	-	-		+	-
Lactobacillus paracasei	+	-	+	+	-
Lactobacillus kitasatonis	-	+	+	+	+
Lactobacillus gallinarum	+	+	-	-	-
Lactobacillus crispatus	+	+	+		+
Lactobacillus plantarum	+	-	+	+	+
Lactobacillus gasseri	+	-	+	+	+

"+" present ; "-" absent

5.1.3 Isolation of Bifidobacterium strains from buttermilk

The sequence obtained after DNA sequencing and amplification was compared with existing sequences in the NCBI database using the Blast N programme. The indents and accession numbers are presented in the Table 5.7. The DNA sequence of the sample matched best with that of *Bifidobacterium pseudocatenulatum strain B1279* and *Bifidobacterium catenulatum strain DSM 16992* with 99% indents.

Table 5.7 Bifidobacterium strains isolated from buttermilk

Strain identified	Indents	Accession
Bifidobacterium pseudocatenulatum strain B1279	99%	NR_037117.1
Bifidobacterium catenulatum strain DSM 16992	99%	NR_041875.1
Bifidobacterium angulatum strain B677	98%	NR_036853.1
Bifidobacterium kashiwanohense strain HM2-2	98%	NR_112779.1
Bifidobacterium ruminantium strain Ru 687	97%	NR_036857.1
Bifidobacterium merycicum strain JCM 8219	97%	NR_115643.1
Bifidobacterium callitrichos strain AFB22-5	97%	NR_113172.1
Bifidobacterium stercoris strain Eg1	97%	NR_116746.1

5.1.4 Isolation of Lactobacillus strains from buttermilk

The sequence obtained after DNA sequencing and amplification was compared with existing sequences in the NCBI database using the Blast N programme. The indents and accession numbers are presented in the Table 5.8. The DNA sequence of the sample matched best with that of *Lactobacillus acidophilus strain JCM 1132* with 99% indent.

Table 5.8 Lactobacillus strains isolated from buttermilk

Strain identified	Indents	Accession
Lactobacillus acidophilus strain JCM 1132	100%	NR_117812.1
Lactobacillus acidophilus strain NBRC 13951	99%	NR_113638.1
Lactobacillus gallinarum strain JCM 2011	98%	NR_113261.1
Lactobacillus gasseri strain ATCC 33820	98%	NR_324831
Lactobacillus plantarum strain	98%	NR_ 042511.1
Lactobacillus reuteri strain	98%	NR_005913.1
Lactobacillus paracasei strain ATCC 25599	97%	NR_008526.1
Lactobacillus crispatus strain DSM 20584	96%	NR_117063.1
Lactobacillus crispatus strain ATCC 33820	96%	NR_041800.1

5.1.5 Isolation of Bifidobacterium strains from rice *ambil*

The sequence obtained after DNA sequencing and amplification was compared with existing sequences in the NCBI database using the Blast N programme. The indents and accession numbers are presented in Table 5.9. The DNA sequence of the sample matched best with that of *Bifidobacterium stercoris strain Eg1, Bifidobacterium faecale strain CU3-7 and Bifidobacterium adolescentis strain ATCC 15703 with 99% indents.*

Table 5.9 Bifidobacterium strains isolated from rice *ambil*

Strain identified	Indents	Accession
Bifidobacterium stercoris strain Eg1	99%	NR_116746.1
Bifidobacterium faecale strain CU3-7	99%	NR_133982.1
Bifidobacterium adolescentis strain ATCC 15703	99%	NR_074802.1
Bifidobacterium ruminantium strain Ru 687	98%	NR_036857.1
Bifidobacterium catenulatum strain DSM 16992	97%	NR_041875.1
Bifidobacterium kashiwanohense strain HM2-2	96%	NR_112779.1
Bifidobacterium pseudocatenulatum strain B1279	97%	NR_037117.1
Bifidobacterium angulatum strain B677	96%	NR_036853.1

5.1.6 Isolation of Lactobacillus strains from rice *ambil*

The sequence obtained after DNA sequencing and amplification was compared with existing sequences in the NCBI database using the Blast N programme. The indents and accession numbers are presented in Table 5.10. The DNA sequence of the sample matched best with that of *Lactobacillus acidophilus strain JCM 1132* with 100% indent.

Table 5.10 Lactobacillus strains isolated from rice *ambil*

Strain identified	Indents	Accession
Lactobacillus acidophilus strain JCM 1132	100%	NR_117812.1
Lactobacillus acidophilus strain NBRC 13951	99%	NR_113638.1
Lactobacillus acidophilus strain VPI 6032	99%	NR_117062.1
Lactobacillus acidophilus strain BCRC10695	99%	NR_043182.1
Lactobacillus kitasatonis strain JCM 1039	98%	NR_024813.1
Lactobacillus gallinarum strain ATCC 33199	98%	NR_042111.1
Lactobacillus gallinarum strain JCM 2011	98%	NR_113261.1
Lactobacillus crispatus strain DSM 20584	98%	NR_117063.1
Lactobacillus crispatus strain ATCC 33820	98%	NR_041800.1

5.1.7 Isolation of Bifidobacterium strains from pearl millet *ambil*

The sequence obtained after DNA sequencing and amplification was compared with existing sequences in the NCBI database using the Blast N programme. The indents and accession numbers are presented in Table 5.11. The DNA sequence of the sample matched best with that of four strains of *Bifidobacterium bifidum* with 99% indent.

Table 5.11 Bifidobacterium strains isolated from pearl millet *ambil*

Strain identified	Indents	Accession
Bifidobacterium bifidum strain NBRC 100015	99%	NR_113873.1
Bifidobacterium bifidum strain DSM 20456	99%	NR_117764.1
Bifidobacterium bifidum strain KCTC 3202	99%	NR_117505.1
Bifidobacterium thermacidophilum strain P3-14	96%	NR_025672.1
Bifidobacterium thermophilum strain AS 1.2235	96%	NR_104968.1
Bifidobacterium scardovii strain CCUG 13008 A	95%	NR_025452.1
Bifidobacterium pseudocatenulatum strain B1279	95%	NR_037117.1
Bifidobacterium lemurum	95%	NR_135862.1

5.1.8 Isolation of Lactobacillus strains from pearl millet *ambil*

The sequence obtained after DNA sequencing and amplification was compared with existing sequences in the NCBI database using the Blast N programme. The indents and accession numbers are presented in Table 5.12. The DNA sequence of the sample matched best with that of *Lactobacillus acidophilus strain JCM 1132* with 100% indent.

Table 5.12 Lactobacillus strains isolated pearl millet *ambil*

Strain identified	Indents	Accession
Lactobacillus acidophilus strain JCM 1132	100%	NR_117812.1
Lactobacillus acidophilus strain NBRC 13951	99%	NR_113638.1
Lactobacillus casei strain JCM 1134	99%	NR_542561.1
Lactobacillus casei ssp. pseudoplantarum strain	99%	NR_083119.1
Lactobacillus kitasatonis strain JCM 1039	98%	NR_024813.1
Lactobacillus plantarum strain ATCC 14917	98%	NR_ 042511.1
Lactobacillus reuteri strain JCM 1112	98%	NR_005913.1
Lactobacillus paracasei strain ATCC 25599	97%	NR_008526.1
Lactobacillus gasseri strain ATCC 33820	97%	NR_324831.1

5.1.9 Isolation of Bifidobacterium strains from barley *ambil*

The sequence obtained after DNA sequencing and amplification was compared with existing sequences in the NCBI database using the Blast N programme. The indents and accession numbers are presented in Table 5.13. The DNA sequence of the sample matched best with that of *Bifidobacterium faecale strain CU3-7, Bifidobacterium stercoris strain Eg1 and Bifidobacterium adolescentis strain ATCC 15703* with 99% indent.

Table 5.13 Bifidobacterium strains isolated from barley *ambil*

Strain identified	Indents	Accession
Bifidobacterium faecale strain CU3-7	99%	NR_133982.1
Bifidobacterium stercoris strain Eg1	99%	NR_116746.1
Bifidobacterium adolescentis strain ATCC 15703	99%	NR_074802.1
Bifidobacterium ruminantium strain Ru 687	98%	NR_036857.1
Bifidobacterium kashiwanohense strain HM2-2	97%	NR_112779.1
Bifidobacterium pseudocatenulatum strain B1279	97%	NR_037117.1
Bifidobacterium catenulatum strain DSM 16992	97%	NR_041875.1
Bifidobacterium angulatum strain B677	96%	NR_036853.1

5.1.10 Isolation of Lactobacillus strains from barley *ambil*

The sequence obtained after DNA sequencing and amplification was compared with existing sequences in the NCBI database using the Blast N programme. The indents and accession numbers are presented in Table 5.14. The DNA sequence of the sample matched best with that of *Lactobacillus acidophilus strain JCM 1132* with 100% indent.

Table 5.14 Lactobacillus strains isolated from barley *ambil*

Strain identified	Indents	Accession
Lactobacillus acidophilus strain JCM 1132	100%	NR_117812.1
Lactobacillus crispatus strain DSM 20584	98%	NR_117063.1
Lactobacillus crispatus strain ATCC 33820	98%	NR_041800.1
Lactobacillus acidophilus strain VPI 6032	99%	NR_117062.1
Lactobacillus kitasatonis strain JCM 1039	98%	NR_024813.1
Lactobacillus plantarum strain ATCC 14917	98%	NR_ 042511.1
Lactobacillus reuteri strain	98%	NR_005913.1
Lactobacillus paracasei strain ATCC 25599	97%	NR_008526.1
Lactobacillus gasseri strain ATCC 33820	97%	NR_324831

5.1.11 Isolation of Bifidobacterium strains from finger millet *ambil*

The sequence obtained after DNA sequencing and amplification was compared with existing sequences in the NCBI database using the Blast N programme. The indents and accession numbers are presented in Table 5.15. The DNA sequence of the sample matched best with that of *Bifidobacterium animalis strain YIT 4121* with 99% indent.

Table 5.15 Bifidobacterium strains isolated from finger millet *ambil*

Strain identified	Indents	Accession
Bifidobacterium animalis subsp. lactis strain YIT 4121	99%	NR_040867.1
Bifidobacterium animalis strain JCM 1190	98%	NR_043438.1
Bifidobacterium pseudolongum strain RU 224	96%	NR_043441.2
Bifidobacterium choerinum strain Su 806	96%	NR_037116.1
Bifidobacterium pseudolongum strain PNC-2-9G	96%	NR_043442.2
Bifidobacterium gallicum strain P6	95%	NR_037118.1
Bifidobacterium cuniculi strain AS 1.2239	95%	NR_104967.1
Bifidobacterium animalis strain ATCC 25527	92%	NR_119007.1
Bifidobacterium breve strain DSM 20213	93%	NR_040783.1

5.1.12 Isolation of Lactobacillus strains from finger millet *ambil*

The sequence obtained after DNA sequencing and amplification was compared with existing sequences in the NCBI database using the Blast N programme. The indents and accession numbers are presented in Table 5.16. The DNA sequence of the sample matched best with that of *Lactobacillus acidophilus strain JCM 1132* with 100% indent.

Table 5.16 Lactobacillus strains isolated from finger millet *ambil*

Strain identified	Indents	Accession
Lactobacillus acidophilus strain JCM 1132	100%	NR_117812.1
Lactobacillus acidophilus strain NBRC 13951	99%	NR_113638.1
Lactobacillus acidophilus strain VPI 6032	99%	NR_117062.1
Lactobacillus acidophilus strain BCRC10695	99%	NR_043182.1
Lactobacillus kitasatonis strain JCM 1039	98%	NR_024813.1
Lactobacillus plantarum strain ATCC 14917	98%	NR_ 042511.1
Lactobacillus crispatus strain DSM 20584	98%	NR_117063.1
Lactobacillus crispatus strain ATCC 33820	98%	NR_041800.1
Lactobacillus gasseri strain ATCC 33820	97%	NR_324831

Result highlights of Phase II

- *Probiotic microorganisms Lactobacillus and Bifidobacterium were present in all fresh ambils.*
- *Predominant species isolated from Genus Lactobacillus were Lactobacillus acidophilus, Lactobacillus reuteri, Lactobacillus casei and Lactobacillus paracasei.*
- *Predominant species isolated from Genus Bifidobacterium were Bifidobacterium bifidum, Bifidobacterium animalis, Bifidobacterium adolescentis.*
- *Probiotic bacteria from the ambils were isolated and studied upto the strain level, Lactobacillus acidophilus was the most predominant bacteria with 100% indent ratio.*
- *Predominant strain present in buttermilk and all the ambils with 100% homology was Lactobacillus acidophilus strain JCM 1132.*
- *Predominant Bifidobacterium strains isolated from buttermilk were Bifidobacterium pseudocatenulatum strain B1279* and *Bifidobacterium catenulatum strain DSM 16992 and* with 99% homology.
- *Predominant Bifidobacterium strains isolated from rice ambil were Bifidobacterium stercoris strain Eg1, Bifidobacterium faecale strain CU3-7 and Bifidobacterium adolescentis strain ATCC 15703* with 99% homology.
- *Predominant Bifidobacterium strains isolated from pearl millet ambil were Bifidobacterium bifidum NBRC 100015, DSM 20456, KCTC 3202* with 99% homology.
- *Predominant Bifidobacterium strains isolated from barley ambil were Bifidobacterium stercoris strain Eg1 and Bifidobacterium adolescentis strain ATCC 15703* with 99% homology.
- *Predominant Bifidobacterium strains isolated from finger millet ambil were Bifidobacterium animalis strain YIT 4121* with 99% homology.

Discussion

Functional foods are defined as: 'foods that contain some health-promoting component(s) beyond traditional nutrients'. Functional foods are also known as designer foods, medicinal foods, nutraceuticals, therapeutic foods, superfoods, foodiceuticals, and medifoods. In general, the term refers to a food that has been modified in some way to become 'functional'. One way in which foods can be modified to become functional is by the addition of probiotics and/or prebiotics (Begum *et al*, 2017). A very promising area in the development of enhanced functional food ingredients is the development of synbiotics which are combinations of a probiotic and a prebiotic (Tadesse, 2012).

New food products have been formulated using fermentation technology which leads to development of probiotic bacteria in foods. Addition of prebiotics enhances the bacterial growth and survival of probiotic bacteria in fermented milks (Sendra *et al*, 2008). In the present study cereal flours and prebiotic FOS were utilized to enhance probiotic potential of the cereal buttermilk drink ambil during fermentation.

Indigenous food products have shown to develop different genus of bacteria which may have probiotic strains. Idli is a natural fermented food, both bacteria and yeasts are generally introduced by the ingredients. Lactobacillus and Leuconostoc are the most is the most commonly encountered bacterium responsible for the taste and texture in Idli (Kannan *et al*, 2015). Dhokla is similar to idli except that the dal used is Bengal gram (Cicer arietinum). LAB contributes lactic acid and acetoin, imparting sour taste and a pleasant flavour (Ray *et al*, 2016; Aidoo *et al*. 2006). In the present study as well ambils developed had desirable flavours which can be attributed to the strains of probiotic cultures.

LAB are so named for their production of lactic acid during the fermentation of carbohydrates. Lactobacillus and Bifidobacterium constitute the maximum number of lactic acid producing bacteria which are commonly associated with the food fermentation processes (Satish *et al*, 2013).

Indian traditional fermented foods are generally fermented by LAB such as Lactococcus spp. Lactobacillus plantarum, L. pentosus, L. brevis, L. fermentum, L. casei, etc and they are considered as the probiotic source from these foods. Availability of certain specific nutrients such as vitamins, minerals and carbon source and also acidic nature of fruits and vegetables provides a growth medium for fermentation by LAB. Buttermilk is an additional source of LAB in such type of fermented foods. Although there are very few beverages reported in this category, and are very popularly consumed in most parts of India. Blandino *et al*. (2003) reported non-LAB Bacillus sp., in rabdi. According to Shah, 2007 and Chow, 2002 the most popular strains are represented by the following genera: Lactobacillus, Streptococcus, and Bifidobacterium, but other organisms including enterococci and yeasts have also been used as probiotics. Other buttermilk and cereal based fermented foods known to develop Lactobacillus strain during the process of fermentation are dosa, selroti, etc. Dosa is a crispy flat thin pancake which is most popular throughout India. LAB like Lactobacillus fermentum, L. lactis, L. delbrueckii, and Lactobacillus plantarum are predominant groups which develop during the dosa fermentation (Gupta *et al*, 2014). Another fermented food is Selroti, a very popular ethnic rice-based fermented product consumed with almost every meal by dwellers of hill areas in Himachal Pradesh, Sikkim, Darjeeling, Nepal, and Bhutan. The microbes associated with selroti batter fermentation are lactic acid producing microbes which were also found during the probiotic profiling of ambil (Yonzan and Tamang, 2010).

Koozhu is the Tamil name for porridge made from millet. Finger millet, a traditional South Indian weaning food, is also consumed in the fermented form, as koozhu in rural and urban households (Ilango and Antony, 2014). Fermented rice or Pazhaiya soru is prepared by adding water to cooked rice and by incubating the mixture overnight, and finally adding buttermilk and salt and directly consumed (Sekar and Mariappan 2007). It is an early morning diet for farmers prior to going to the field. Predominant microbiota isolated from these foods include: Lactobacillus fermentum, L. plantarum and. L. plantarum (Satish *et al*. 2010).

Lactobacillus is a major bacterium that has been found in almost all the fermented food products mentioned above, and so has been found in all the ambils. Other fermented food products which have been documented to develop Lactobacillus species during the process of fermentation are *haria* from East central India (Ghosh, 2014), *apong* from Assam and Arunachal Pradesh (Das *et al*, 2012), jou and judima from North-east India (Chakrabarty *et al*, 2014), *zuth*o from Nagaland (Teramoto, *et al* 2014), etc

The *ambils* developed in this study were studied for their probiotic potential and all the *ambils* had shown the growth of beneficial probiotic bacteria of Lactobacillus and Bifidobacterium strains during the fermentation process. Similar studies conducted on Dadih, an indigenous fermented milk based drink of Indonesia had documented presence of strains Lactobacillus reuteri IS-27560, Lb. plantarum IS-10506 and Lb. rhamnosus IS-7257 upon isolation (Collado *et al*, 2007). Lb.acidophilus Lb. rhamnosus FSMM15, FSMM22, FSMM26 were isolated from a fermented mare milk (Antara *et al*, 2009; Shi *et al*, 2012).

Kefir is a fermented milk product originating from the Northern Caucasus, Lactobacillus was isolated and isolates were identified by amplified ribosomal DNA restriction analysis and 16S rDNA sequencing of representative amplicons. The strains present were Lactobacillus paracasei MRS59, M1743 and MRS55 (Leite *et al*, 2015). The results of our study and those of previous studies imply that the homology levels between some Lactobacillus strains reached more than 99% (Ritchie *et al*. 2010).

Lactic acid bacteria were isolated from traditional yogurt samples and genotypic characterization of these isolates revealed the presence of 21 distinct LAB strains including Lactobacillus reuteri LB 121, Lactobacillus plantarum Y22, Y36, Y48, Y69, etc. The present study had also documented presence of Lactobacillus strains of Lb. reuteri and Lb plantarum (Ispirli and Dertli, 2018).

Thirty-two replicate samples of indigenous Dahi from Nepal and were studied for probiotic organisms. A total of 120 isolates of Lactobacillus were obtained. Predominant strains isolated were Lactobacillus delbrueckii RHL 77, RHL 89, LHL 128 etc, Lactobacillus paracasei GHM 12, GHM 66, LHL 133, etc (Koirala *et al* 2014). In another similar study, nineteen samples of commercially manufactured yoghurts samples were evaluated for total viable count of Lactic Acid Bacteria (LAB) using selective media. Identified Lactobacillus species include L. plantarum, strains M18A, M12A, M14B, Y19B, Y-GW, etc (Khalil and Anwar, 2016).

LAB strains from traditional Iranian dairy products using 16S rDNA gene sequencing. A total of 200 samples of traditional fermented dairy products including yogurt, curd, shiraz and tarkhineh from west of Iran were selected and subjected to analysis. Strains isolated were L. paracasei subsp. paracasei 46Lac, L. plantarum 15HN, L. lactis subsp. lactis 44Lac and L. lactis subsp. cremoris 44L (Haghshenas *et al* 2017).

The biodiversity of Lactobacillus species in fermented dairy products is variable and region specific. In traditional Spanish cheese (Armada cheese), the predominant Lactobacilli are L. casei subsp. casei and L. brevis (Castro *et al*. 2015). Meanwhile, in Greek goat cheese (Batzos cheese), L. paracasei and L. sakei are the dominant species (Cárdenas *et al*. 2014), whereas in Brazilian fresh cheese (Minas Frescal cheese) the predominant species is L. acidophilus (Lollo *et al*. 2012). All the strains mentioned revealed that the results of the present are quite different from those in terms of strains and prevalence (Haghshenas *et al*, 2017).

Zuo *et al*, 2014, had isolated similar strains were isolated from traditional dairy products cheese, koumiss and raw milk. Similar results were also documented by Liu *et al*. 2012 and Watanabe *et al*. 2008 in other types of natural dairy foods. It may suggest that a high viability of LAB in the traditional dairy products, which is an important requirement for further functional food development. Therefore, it may useful to further characterize the microbial diversity and composition of these conventional dairy products.

Dewan and Tamang (2007) conducted a research on 58 samples of Himalayan ethnic fermented milk products and a total of 128 isolates of LAB were isolated and classified into 3 genera and 10 species and subspecies.

A previous study (Zamfir *et al*., 2006) on the LAB diversity in sour cream of Romanian found that the predominant species were L. lactis. Torres Llanez *et al*. (2006) conducted a research on artisanal Mexican Fresco cheese, they concluded that the predominant LAB were L. lactis, and L. casei. Bulut *et al*. (2005) carried out a research on the traditional comek peynir cheese from the Cappadocia region and reported L. paracasei as the prevalent bacteria.

The fermenting microorganisms which were successfully isolated from all the ambils were characterized by morphological, colonial, biochemical as well as molecular characteristics. During fermentation, lactic acid bacteria contribute to sour taste due to increase in the acidity of the product and improve the flavour. Also, The Lactobacillus and Bifidobacterium species found in the *ambils* are all known to have many therapeutic and health beneficial properties (Beerepoot, 2012; Singh, 2013; Singhi and Kumar, 2016; *Schreck et al, 2017;* Nie *et al*, 2015; Park *et al*, 2015). Similar to *Lactobacillus*, *Bifidobacterium* species isolated from ambils are all known to have health beneficial therapeutic properties *(*Jungersen *et al*, 2014; Merenstein *et al*, 2015). Thus it can be concluded that fermentation, an age old technique, leads to the development of beneficial bacteria during the process and can also be considered as a functional food.

Conclusion

- *Some of the LAB reported in these fermented foods are demonstrated to confer a beneficial effect to the host, in another sense they possess probiotic properties.*
- *Thus, Ambils can be considered as probiotic beverage as it contains species of Lactobacillus and Bifidobacterium thus can be considered as a therapeutic beverage because of the many health beneficial probiotic bacteria present.*
- *There is an abundant opportunity available for food microbiologists to explore the Indian fermented foods for the isolation of new LAB strains for their potential role in probiotic research.*

Phase III - Physicochemical properties, nutritional composition of buttermilk and ambils and shelf life of fresh and packaged *ambils*.

Functional foods can be considered to be whole, fortified, enriched or enhanced foods that provide health benefits beyond the provision of essential nutrients (e.g., vitamins and minerals), when they are consumed at efficacious levels as part of a varied diet on a regular basis. One of the most important considerations when developing a new food product or reformulating an existing one is to ensure that the food products desired properties, quality and safety are maintained throughout the product entire shelf life and stated storage conditions. Food ingredients can undergo deteriorative changes during their shelf life that can impact on their chemical, sensory and nutritional properties.

Rice ambil was packaged (details of packaging explained in Chapter 3 Methodology) and studied for its physicochemical properties, nutritional composition and shelf life stability along with all fresh ambils and buttermilk. The results of this phase of the study have been divided into following sections:

5.2.1 Physicochemical properties of buttermilk and fresh ambils

5.2.2 Physicochemical properties of packaged rice ambil

5.2.3 Nutritional composition of buttermilk and fresh ambils

5.2.4 Nutritional composition of packaged rice ambil

5.2.5 Changes in nutritional composition before and after UHT treatment and packaging

5.2.6 Shelf life of fresh ambils

5.2.6.1 Organoleptic evaluation, physicochemical properties and microbial changes in rice ambil

5.2.6.2 Organoleptic evaluation, physicochemical properties and microbial changes in pearl millet ambil

5.2.6.3 Organoleptic evaluation, physicochemical properties and microbial changes in barley ambil

5.2.6.4 Organoleptic evaluation, physicochemical properties and microbial changes in finger millet ambil

5.2.7 Shelf life of packaged rice ambils

5.2.7.1 Organoleptic evaluation

5.2.7.2 Physicochemical properties

5.2.1 Physicochemical properties of buttermilk and fresh ambils

The physicochemical properties of buttermilk and ambils are depicted in Table 5.17, Fig 5.1 (a-d).

5.2.5.1 Moisture

The percent moisture in buttermilk was 95.85, and amongst the various cereal-millet based ambils it was highest in barley *ambil* (87.17) followed by pearl millet *ambil* (86.8), and a significant difference amongst all the ambils was observed.

5.2.5.2 Acidity

Percent acidity of buttermilk was 0.50, whereas amongst the *ambils* barley *ambil* was the highest (0.56), followed by pearl millet *ambil* (0.54) with no significant difference.

5.2.5.3 pH

pH of buttermilk was 4.2, and amongst the various cereal-millet based ambils it was highest in barley *ambil* (4.68) followed by pearl millet (4.58) and rice *ambil (4.56)* with no significant difference.

5.2.5.4 Viscosity

A significant difference (p<0.000) was found amongst all the ambils, rice *ambil* (23.3 mPa.s @20°) was highest as compared to others, followed by barley ambil (22.9 mPa.s @20°).

Table 5.17 Physicochemical properties of fresh ambils

Parameters	Rice ambil	Barley ambil	Pearl millet ambil	Finger millet ambil	p-value
Moisture (%)	84.87 ±0.25	87.17 ±0.25	86.80 ±0.36	84.70 ±0.20	0.000
pH	4.56 ±0.45	4.68 ±0.31	4.58 ±0.35	4.04 ±0.23	0.574
Acidity (%)	0.51 ±0.03	0.56 ±0.03	0.54 ±0.06	0.51 ±0.02	0.378
Viscocity (mPa.s @20°.)	23.3 ±0.20	22.9 ±0.20	21.53 ±0.25	19.57 ±0.35	0.000

- Mean values represent the average of 10 determinants in triplicate
- Level of significance in increasing order – (*p<0.05, **p<0.01, ***p<0.001)

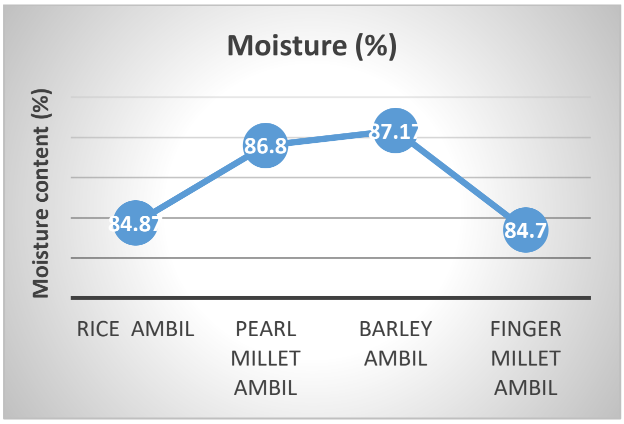

Fig 5.1 (a) Moisture content in fresh ambils

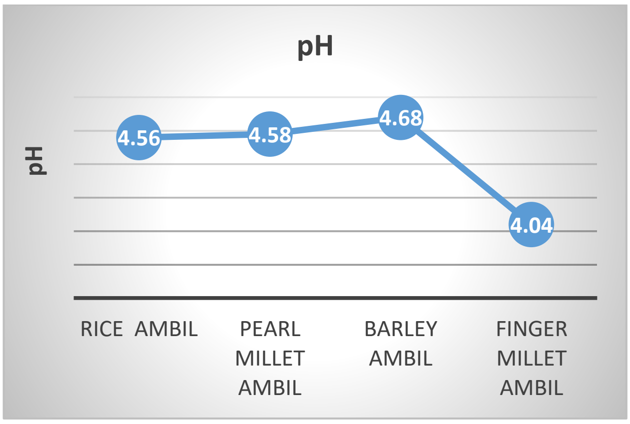

Fig 5.1 (b) pH content in fresh ambils

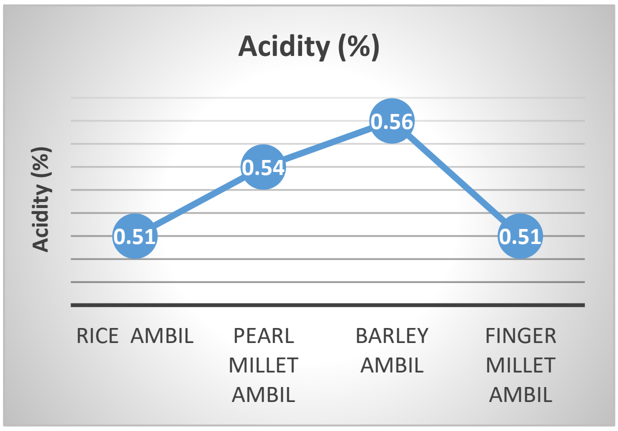

Fig 5.1 (c) Acidity in fresh ambils

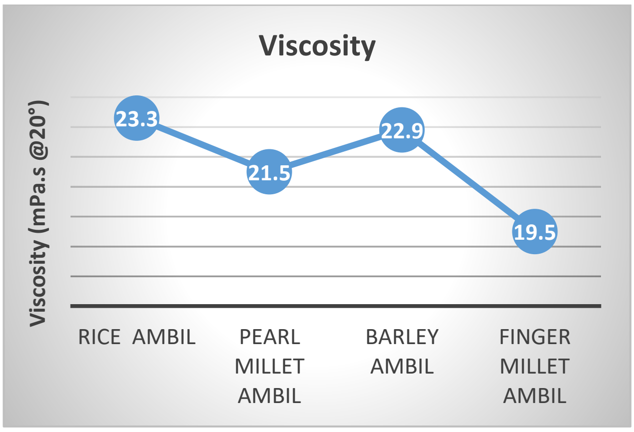

Fig 5.1 (d) Viscosity of fresh ambils

Fig 5.1 (a-d) Physicochemical properties of fresh ambils

5.2.2 Physicochemical properties of packaged rice ambil

As seen in Table 5.18 viscosity of packaged rice ambil was 21.8 mPa.s @20° whereas the acidity and pH content was 0.36% and 4.6 respectively.

Table 5.18 Physicochemical properties of packaged ambil (per 100ml)

Parameters	Rice ambil packaged
Moisture (%)	83.6 ±2.38
pH	4.6 ±0.45
Acidity (%)	0.36 ±0.01
Viscocity (mPa.s @20°.)	21.8 ±1.01

Values are mean ± SD (n = 3)

5.2.3 *Nutritional composition of buttermilk and fresh ambils*

Nutritional composition of buttermilk and fresh ambils was analyzed for proximate principles, total calorie, crude fibre, vitamins and minerals. The results are depicted in Table 5.19, Table 5.20 and Fig 5.2 (a-s)

5.2.3.1 Carbohydrates

Carbohydrate content in buttermilk was 0.5 g per 100 ml. A significant difference was found between the ambils, highest in rice *ambil* (6.4 g) followed by finger millet *ambil* (5.95 g).

5.2.3.2 Protein

The protein content in buttermilk was 0.8 g per 100 ml. A significant difference was found between the ambils ambils, with highest in barley ambil (1.22 g) and pearl millet *ambil* (1.22 g), followed by rice *ambil (1g)*.

5.2.3.3 Total fat

The fat content of buttermilk was 1.3 g per 100 ml. A significant difference was found between the ambils, highest in finger millet ambil (0.9 g) followed by barley ambil (0.5 g). Rice ambil and pearl millet ambil had similar fat content (0.45 g). No significant difference was observed for saturated fats and MUFA amongst the ambils.

5.2.3.4 Calories

The total calorie content in buttermilk was 15 kcal in 100 ml. A significant difference was found between the ambils, with highest in pearl millet ambil (34.5 kcal) followed by rice ambil (33.3 kcal) and barley ambil (32.5 kcal). Ragi ambil had the least calorie content.

5.2.3.5 *Crude fiber*

A significant difference for the crude fiber content was found between the ambils, highest in barley ambil (0.32 g) and least in rice ambil (0.01 g).

5.2.3.6 Total ash

Total ash content of buttermilk in percentage was 0.18. A significant difference was found between the ambils, highest in pearl millet *ambil* (0.73 %) followed by rice *ambil* (0.67 %).

5.2.3.7 Vitamins

The folic acid content of pearl millet ambil (8.43 mcg) was determined as highest, followed by finger millet ambil (3.10 mcg) and barley ambil (2.54 mcg). Vitamin A content was highest in finger millet ambils (53 IU) whereas Vitamin B1 and Vitamin B3 content was highest in barley ambil. However the pearl millet ambil was found to have highest Vitamin B2.

5.2.3.8 Minerals

Calcium content of finger millet *ambil* was 38.9 mg per 100ml, highest amongst all ambils followed by pearl millet ambil (14.7). Sodium content of all the ambils were approximately similar and varied from 327 mg to 329 mg. However potassium content varied amongst all ambils in the range of 60.7 mg to 123.8 mg, highest in barley ambil, and least in rice ambil. Phosphorus content was seen highest in pearl millet *ambil* (35 mg) followed by finger millet *ambil* (34 mg).

Table 5. 19 Nutritional composition of buttermilk (per 100ml)

Parameters	**Buttermilk**		**Parameters**	**Buttermilk**
Protein (g)	0.8 ±0.06		Total Ash (%)	0.18 ±0.25
CHO (g)	0.5 ± 0.65		Vitamin A (IU)	51.15 ±0.44
Calories (kcal)	15 ± 1.12		Magnesium (mg)	26.95 ± 1.2
Total fat (g)	1.3 ± 0.12		Calcium (mg)	30 ±0.92
Saturated fat (g)	1.04 ± 0.27		Sodium (mg)	105.25 ±0.25
MUFA (g)	0.22 ± 0.24		Potassium (mg)	151.18 ±1.25
PUFA (g)	0.08 ± 0.02		Phosphorus (mg)	30 ±0.86

Values are mean ± SD (n = 3)

Table 5.20 Nutritional composition of fresh ambils (per 100ml)

Parameters	Rice Ambil	Pearl millet Ambil	Barley Ambil	Finger millet Ambil	p Value
Protein (g)	1 ±0.14	1.22 ±0.21	1.22 ±0.26	0.88 ±0.31	0.005
CHO (g)	6.4 ±0.28	5.59 ±0.21	5.75 ±0.21	5.95 ±0.15	0.003
Calories (kcal)	33.3 ±0.40	34.5 ±0.17	32.5 ±0.20	31.9 ±0.50	0.001
Crude fibre (g)	0.01 ±0.15	0.1 ±0.06	0.32 ±0.06	0.29 ±0.06	0.000
Total Ash (%)	0.67 ±0.21	0.73 ±0.15	0.60 ±0.26	0.63 ±0.21	0.007
Total fat (g)	0.45 ±0.17	0.45 ±0.21	0.52 ±0.10	0.9 ±0.11	0.000
Saturated fat (g)	0.17 ±0.06	0.20 ±0.10	0.20 ±0.10	0.20 ±0.10	0.76
MUFA (g)	0.13 ±0.06	0.13 ±0.06	0.17 ±0.06	0.17 ±0.06	0.20
PUFA (g)	0.05 ±0.02	0.13 ±0.06	0.03 ±0.06	0.03 ±0.06	0.000
Folic acid (mcg)	2.07 ±0.29	8.43 ±0.47	2.54 ±0.04	3.10 ±0.70	0.000
Vitamin A (IU)	43 ±0.06	47 ±0.15	47 ±0.25	53 ±0.17	0.002
B1(mcg)	0.01 ±0.01	0.05 ±0.02	0.08 ±0.01	0.07 ±0.03	0.000
B2(mcg)	0.01 ±0.01	0.05 ±0.02	0.04 ±0.01	0.04 ±0.03	0.016
B3(mcg)	0.09 ±0.02	0.03 ±0.01	0.91 ±0.03	0.06 ±0.04	0.000
Magnesium (mg)	16.30 ±1.04	25.27 ±0.21	3.60 ±0.20	24.80 ±0.95	0.000
Calcium (mg)	12.2 ±0.26	14.70 ±0.17	13.4 ±0.26	38.9 ±0.06	0.000
Sodium (mg)	327 ±0.42	328 ±0.15	329 ±0.20	328 ±0.62	0.69
Potassium (mg)	60.7 ±0.55	81.8 ±0.40	123.8 ±0.32	62.2 ±0.44	0.000
Phosphorus (mg)	24.2 ±0.25	35 ±0.51	28.6 ±0.25	34 ±0.87	0.000

- Values are mean ± SD (n = 3)
- Level of significance in increasing order – (*p<0.05, **p<0.01, ***p<0.001)

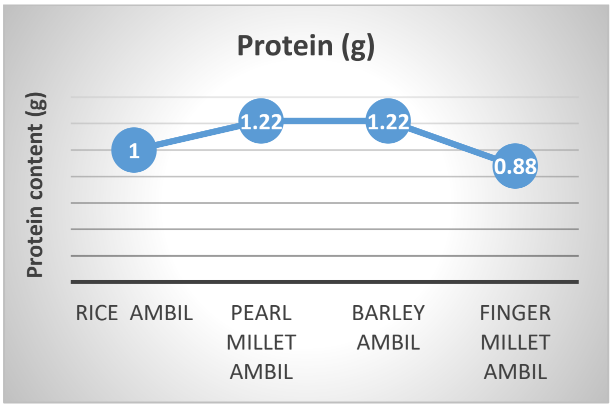

Fig 5.2 (a) Protein content in fresh ambils

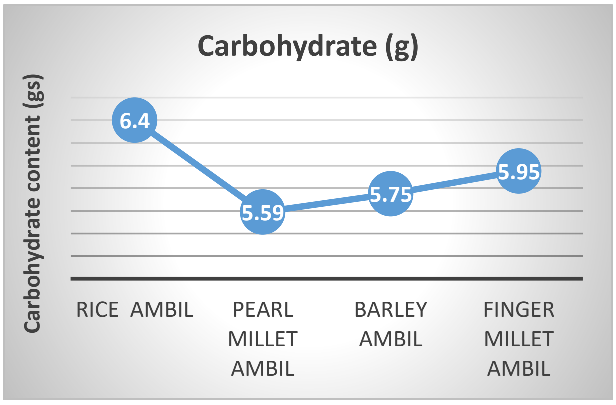

Fig 5.2 (b) Carbohydrate content in fresh ambils

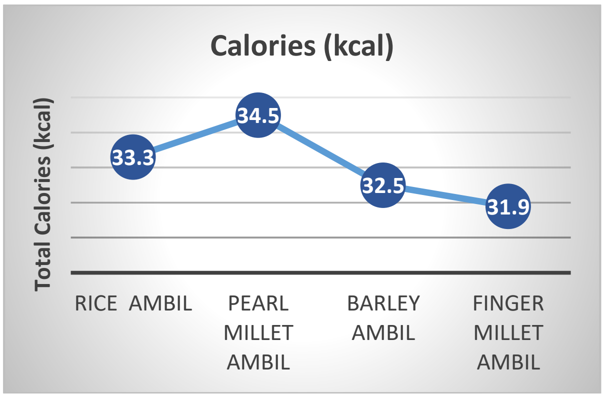

Fig 5.2 (c) Total calories in fresh ambils

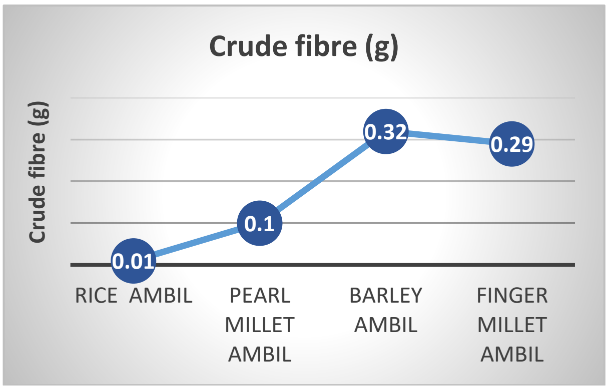

Fig 5.2 (d) Crude fibre content in fresh ambils

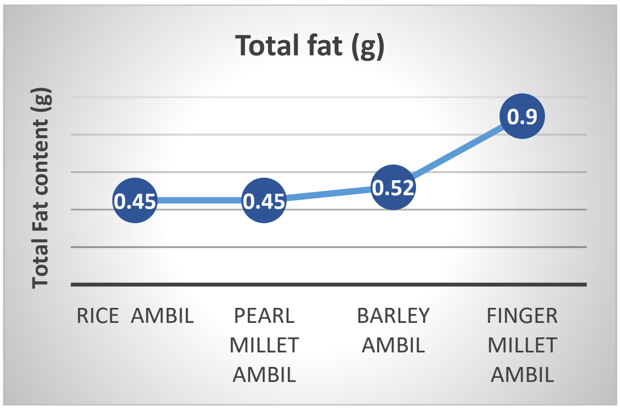

Fig 5.2 (e) Total fat content in fresh ambils

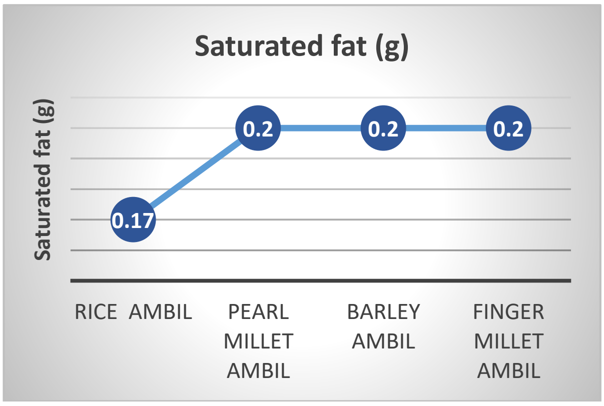

Fig 5.2 (f) Saturated fat content in fresh ambils

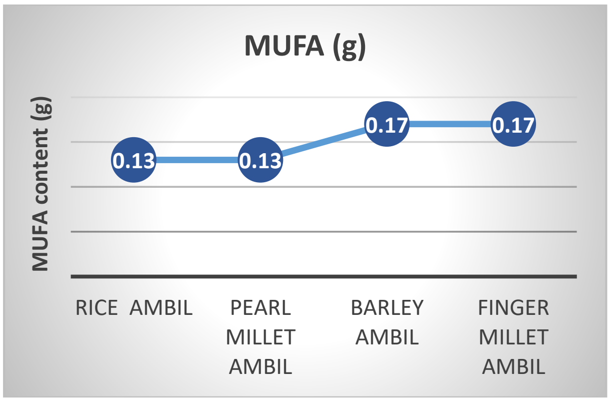

Fig 5.2 (g) MUFA content in fresh ambils

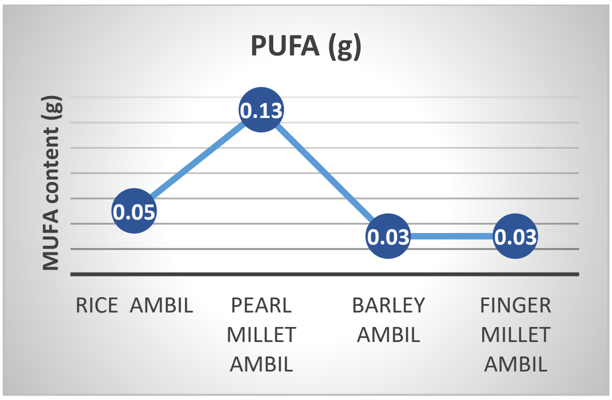

Fig 5.2 (h) PUFA content in fresh ambils

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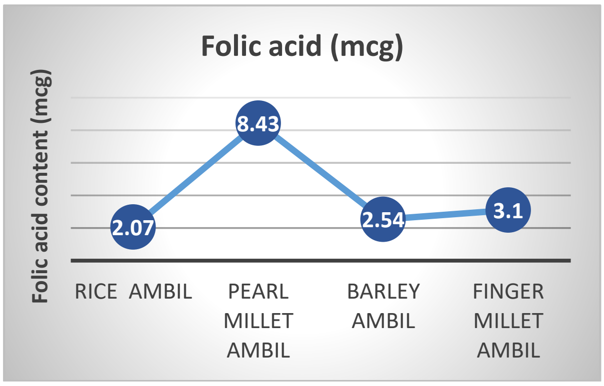

Fig 5.2 (i) Folic acid content in fresh ambils

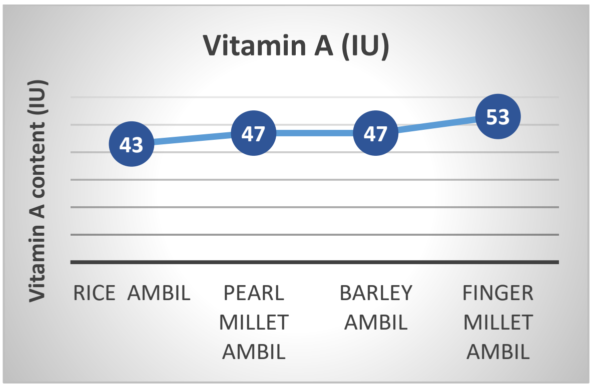

Fig 5.2 (j) Vitamin A content in fresh ambils

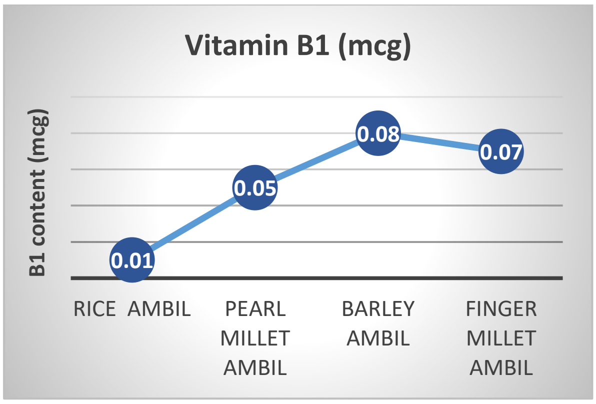

Fig 5.2 (k) Vitamin B1 content in fresh ambils

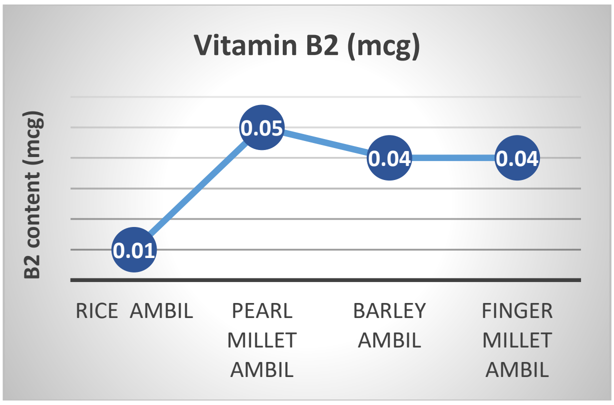

Fig 5.2 (l) Vitamin B2 content in fresh ambils

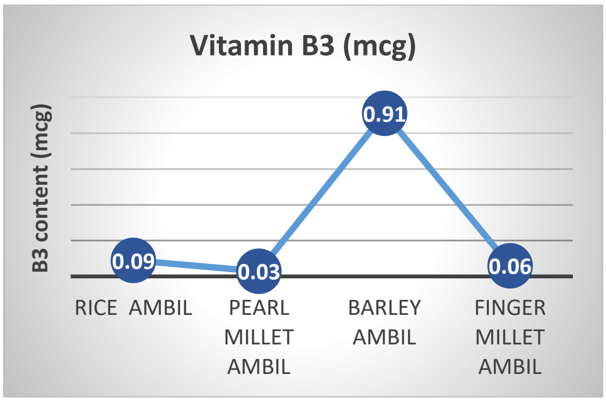

Fig 5.2 (m) Vitamin B3 content in fresh ambils

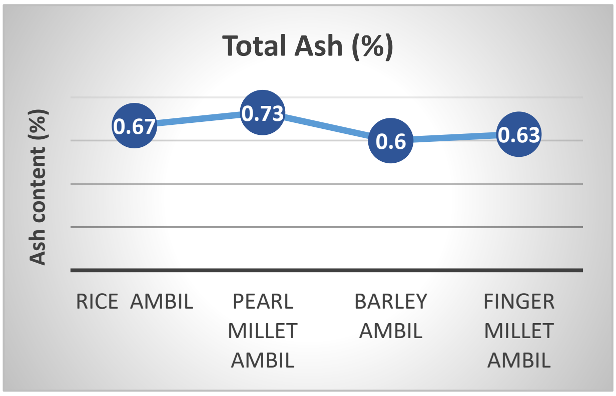

Fig 5.2 (n) Total ash content in fresh ambils

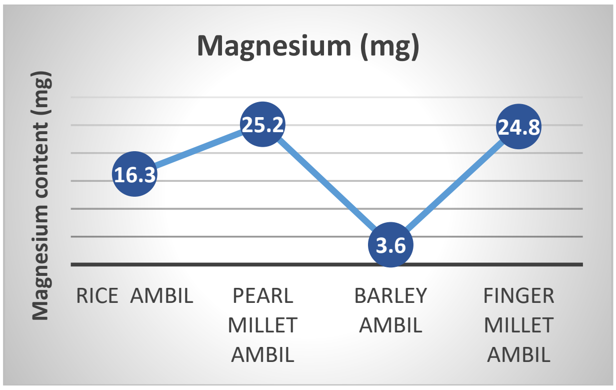

Fig 5.2 (o) Magnesium content in fresh ambils

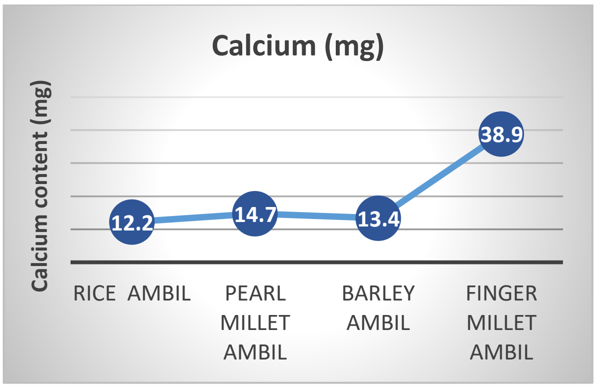

Fig 5.2 (p) Calcium content in fresh ambils

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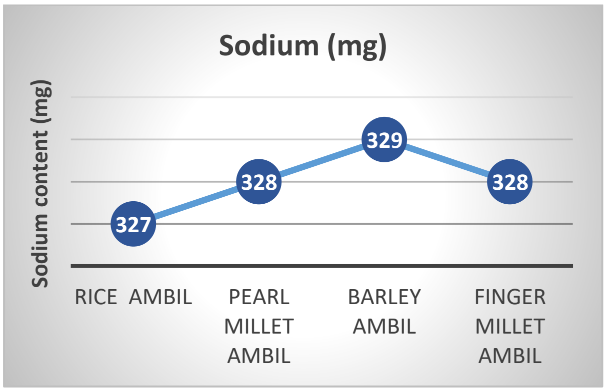


Fig 5.2 (q) Sodium content in fresh ambils

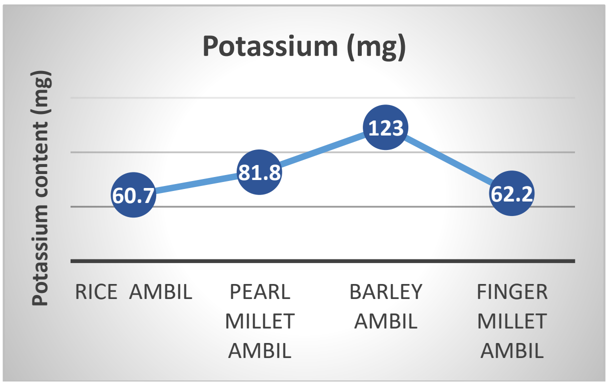


Fig 5.2 (r) Potassium content in fresh ambils

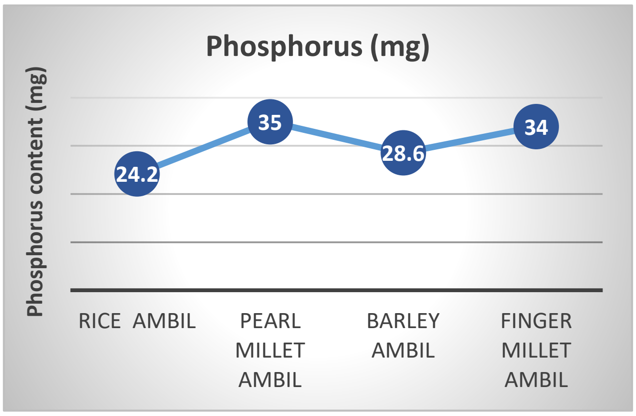


Fig 5.2 (s) Phosphorus content in fresh ambils

Fig 5.2 (a-s) Nutritional composition of ambils

5.2.4 Nutritional composition of packaged rice ambil

The nutritive value of packaged rice ambil depicted in Table 5.21 reveals that it’s a low calorie and low fat (0.49 g) beverage with moderate calcium (84.6 g) and protein (1.2 g) content in 100 ml. Fiber content could not be analyzed as the amount was low than desired levels. Moisture and total ash was determined as 83.6% and 0.9% respectively. Vitamin A and Vitamin B1 was found to be 48IU and 0.18 mcg respectively. Other vitamins were in negligible traces. Moderate amounts of minerals were observed.

Table 5.21 Percentage difference for Nutritional composition between fresh and packaged rice ambil (per 100ml)

Parameters	Fresh rice ambil	Packaged rice ambil	% difference
Moisture (%)	84.87 ±2.12	83.6 ± 2.38	-0.01
Protein (g)	1 ±0.14	1.2 ±0.01	0.20
CHO (g)	6.4 ±0.28	8.4 ±0.34	0.31
Calories (kcal)	33.3 ±0.40	41.2 ±0.21	0.24
Total Ash (%)	0.67 ±0.21	0.9± 0.58	0.34
Total fat (g)	0.45 ±0.17	0.49± 0.02	0.08
Saturated fat (g)	0.17 ±0.06	0.27±0.07	0.59
MUFA (g)	0.13 ±0.06	0.11±0.01	-0.15
Magnesium (mg)	16.30 ±1.04	49± 0.11	2.01
Calcium (mg)	12.2 ±0.26	84.6± 1.23	5.93
Sodium (mg)	327 ±0.42	238.1± 1.86	-0.27
Potassium (mg)	60.7 ±0.55	33.4± 1.12	-0.45
Phosphorus (mg)	24.2 ±0.25	30.4± 2.38	0.26
Vitamin A (IU)	43 ±0.06	48± 1.23	0.12
Vitamin B1 (mcg)	0.01 ±0.01	0.01± 0.01	0
Vitamin B2 (mcg)	0.01 ±0.01	0.01 ±0.01	0
Vitamin B3 (mcg)	0.09 ±0.02	0.05 ±0.02	-0.44
Folic acid (mcg)	2.07 ±0.29	1.98 ±0.18	-0.04

Values are mean ± SD (n = 3)

5.2.5 Changes in nutritional composition before and after UHT treatment and packaging

Packaging of rice ambil involved UHT treatment, wherein it is expected that all the microbes are killed and some nutritional losses may have taken place. As seen in Table 5.21 it can be observed that some nutrients increased during the UHT treatment applied before packaging the ambil. However, some nutrition losses can also be seen. Slight reduction in moisture content (-0.01%) was observed. Vitamin B3 and folic acid had also showed slight reduction after the treatment, however no change was observed in Vitamin B1 and Vitamin B2 content. The nutrients which increased after packaging were fat (0.08%), calcium (5.93%) and magnesium (2.01%).

5.2.6 Shelf life of fresh ambils

All the freshly prepared *ambils* were stored in refrigerator at 4°C ±1 in glass bottles with a tight fitting lid. The storage stability was studied for a period of 5 days, or till the product became unacceptable in terms of its physical appearance and aroma. Organoleptic characteristics and physical and microbial parameters were studied including pH, acidity, viscosity, peroxide value, whey separation, and microbial parameters including TPC, Coliform, yeast and mold.

5.2.6.1 Organoleptic evaluation, physicochemical properties and microbial content of rice ambils

(a) Organoleptic evaluation of freshly prepared rice ambils

Freshly prepared rice *ambil* was stored in refrigerator at 4°C ±1, there was no significant ($p<0.05$) difference in color and appearance during the storage period of 5 days, but 4% reduction of scores was observed from day 1 to day 5 (Table 5.22 and Fig 5.3). Consistency changed significantly ($p<0.000$) during the storage period which decreased to 9% on day five. Aroma scores showed a significant difference ($p<0.000$) from day one (8.6) to the end of five days (7.0), likely due to increase in acidity. No significant difference in taste and flavour was observed upto 3 days, however after 3rd day there was slight reduction (31%) in the scores of taste and flavour, but still the product was graded in fairly acceptable category. The reduction was significant from day 3 to day 5 (p<0.000), similarly no significant difference in mouthfeel was seen till day 3, but decreased after that (31%). Overall acceptability scores did not show any significant decrease till day 3 but gradually decreased by day 5, and the change was significant (p<0.000). Thus it can be concluded that overall there was no significant reduction in the scores till day 3, also the product was scored in fairly acceptable category even at the day 5.

Table 5.22 Mean scores for the sensory attributes of fresh Rice *ambil* over a storage period of 5 days

Parameters / Days	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	p – value	%age difference
Color	8.9 ±0.31	8.8 ±0.41	8.7 ±0.47	8.7 ±0.45	8.5 ±0.5	8.5 ±0.5	0.029^{NS}	-4%
Consistency	8.8 ^{a} ±0.41	8.8 ^{a} ±4.4	8.7 ^{a} ±0.47	8.4 ^{ab} ±0.4	8.13 ^{b} ±0.73	8 ^{b} ±0.6	0.000	-9%
Aroma	8.6 ^{a} ±0.5	8.6 ^{a} ±0.5	8.2 ^{ab} ±0.73	7.6 ^{b} ±0.99	7.1 ^{b} ±1.32	7 ^{b} ±1.36	0.000	-19%
Taste n flavour	8.7 ^{a} ±0.47	8.7 ^{a} ±0.47	8.5 ^{a} ±0.5	7.3 ^{c} ±0.65	6.4 ^{b} ±0.5	6^{b} ±0.85	0.000	-31%
Mouth feel	8.8 ^{a} ±0.41	8.8 ^{a} ±0.41	8.3 ^{b} ±0.65	7.6 ^{c} ±1	6.1 ^{d} ±0.38	6 ^{d} ±0.25	0.000	-31%
After taste	8.4 ^{a} ±0.5	8.1 ^{a} ±0.31	7.7 ^{b} ±0.47	6.9 ^{c} ±0.89	6.4 ^{d} ±0.5	5.9 ^{e} ±0.25	0.000	-25%
Absence of defects	8.2 ^{a} ±0.45	8.1 ^{a} ±0.38	7.6 ^{b} ±0.61	7 ^{c} ±1.01	6.3 ^{d} ±0.61	6 ^{d} ±0.25	0.000	-25%
OA	8.7 ^{a} ±0.45	8.7 ^{a} ±0.45	8.6 ^{a} ±0.49	8.6 ^{b} ±0.18	6.2 ^{c} ±0.61	5.9 ^{c} ±0.54	0.000	-13%

- Mean values represent the average of 10 determinants in triplicate
- a, b, c – The non-identical letters in any 2 columns within the row denote a significant difference at a minimum of 5% level
- NS – the difference between the mean values within the columns is not significant.
- Level of significance in increasing order – (*p<0.05, **p<0.01, ***p<0.001)

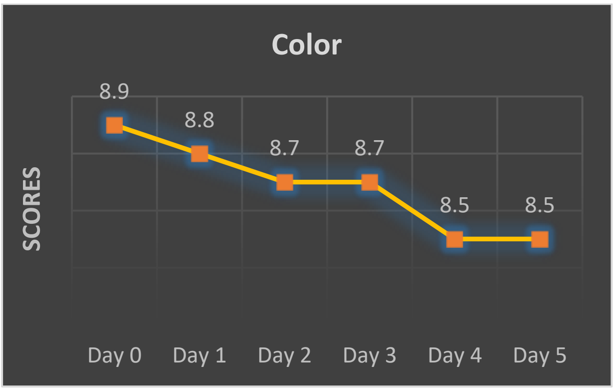


Fig 5.3 (a) Color of fresh ambils

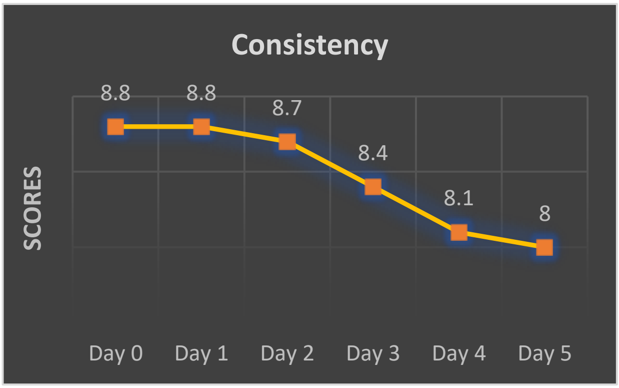


Fig 5.3 (b) Consistency of fresh ambils

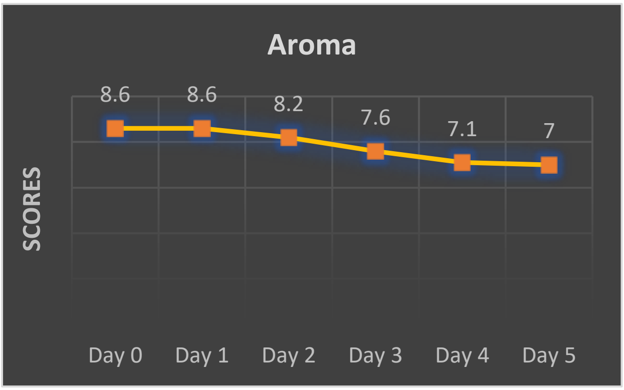


Fig 5.3 (c) Aroma of fresh ambils

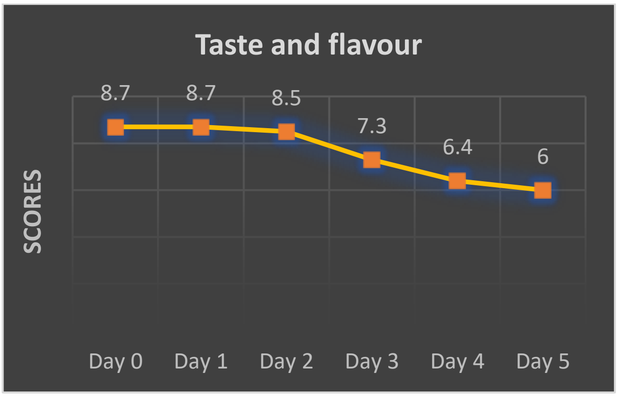


Fig 5.3 (d) Taste and flavour of fresh ambils

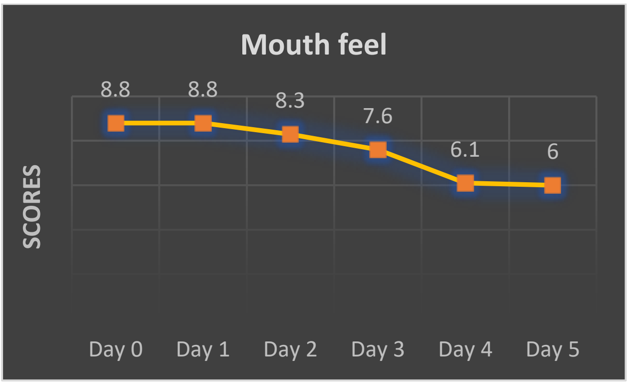


Fig 5.3 (e) Mouthfeel of fresh ambils

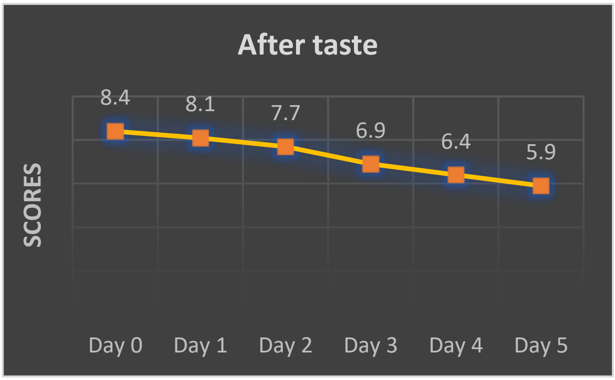


Fig 5.3 (f) After taste of fresh ambils

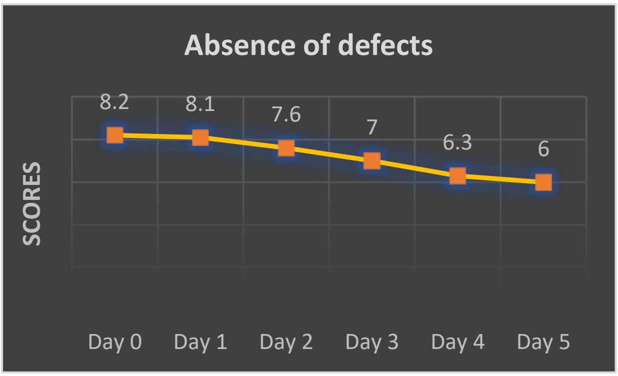


Fig 5.3 (g) Absence of defects in fresh ambils

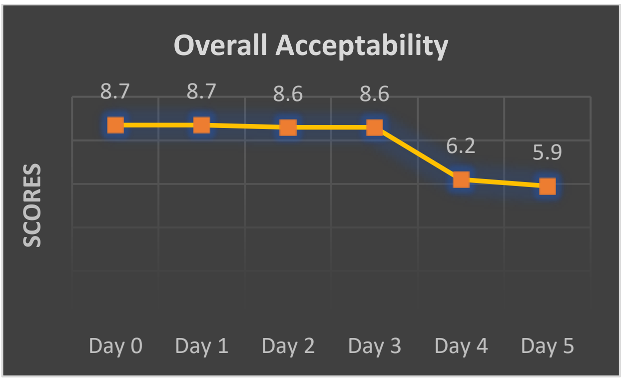


Fig 5.3 (h) Overall acceptability of fresh ambils

Fig 5.3 (a-h) Sensory attributes of fresh Rice ambil over a storage period of 5 days

(b) Physicochemical properties of freshly prepared rice ambils

A gradual decrease in pH and an increase acidity was observed during the storage (Table 5.23 and Fig 5.4 a-e). pH of freshly prepared rice *ambil* was significantly reduced by 0.19% from day 1 to day 5. A significant increase in acidity was also observed over the storage period, ranging from 0.45 on day one to 1.67 on day five of storage (4.04% increase). No change in the peroxide value was observed. Viscosity of rice *ambil* was found to be 23.3 (mPa.s @20°) at day 0, which increased by 0.02% at the end of day 5 (22.3 mPa.s @20°). Whey separation index of different *ambils* was determined (expressed as ml whey expelled/ 10 ml of *ambil*). It gradually reduced by 0.08% over the storage period.

Table 5.23 Mean scores for the physicochemical properties of fresh Rice *ambil* over a storage period of 5 days

Parameters / Days	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	p – value	%age difference
pH	4.9 ^{a} ±0.01	4.7 ^{ac} ±0.06	4.5 ^{a} ±0.12	4.4 ^{ad} ±0.21	4.1 ^{ad} ±0.09	3.9 ^{bcd} ±0.42	0.001	-0.19%
Acidity	0.45 ^{a} ±0.03	0.56 ^{ab} ±0.02	0.73 ^{b} ±0.08	0.83 ^{c} ±0.02	1.09 ^{d} ±0.06	1.47 ^{d} ±0.07	0.000	4.04%
Peroxide value	<10	<10	<10	<10	<10	<10	-	-
Viscosity	21.6 ^{a} ±0.11	21.6 ^{a} ±0.11	22.1 ^{a} ±0.23	22.1 ^{a} ±0.05	22.2 ^{a} ±0.1	22.3 ^{b} ±0.26	0.001	0.02%
Whey separation	1.86 ±0.05	1.76 ±0.05	1.73 ±0.11	1.66 ±0.05	1.63 ±0.11	1.7 ±0.1	0.002^{NS}	-0.08%

- Mean values represent the average of 10 determinants in triplicate
- a, b, c – The non-identical letters in any 2 columns within the row denote a significant difference at a minimum of 5% level
- NS – the difference between the mean values within the columns is not significant.
- Level of significance in increasing order – (*p<0.05, **p<0.01, ***p<0.001)

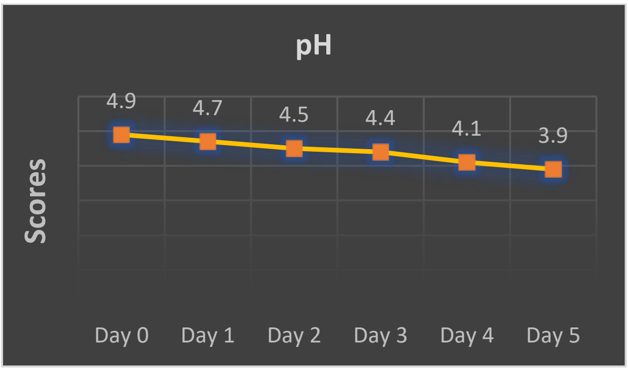


Fig 5.4 (a) pH of fresh ambils

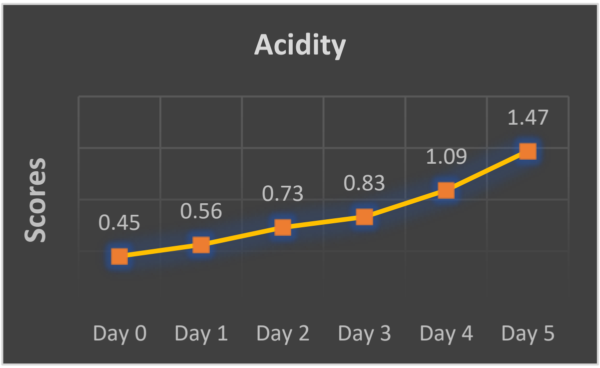


Fig 5.4 (b) Acidity in fresh ambils

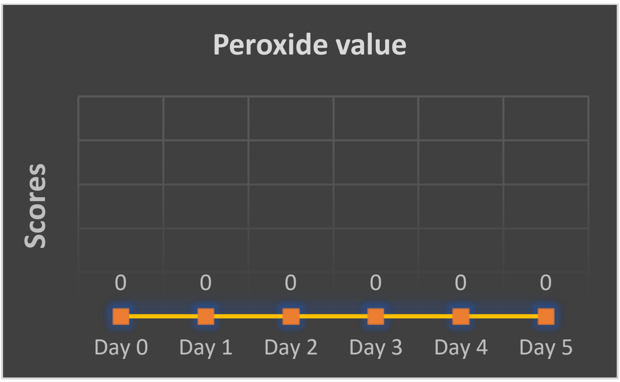


Fig 5.4 (c) Peroxide value of fresh ambils

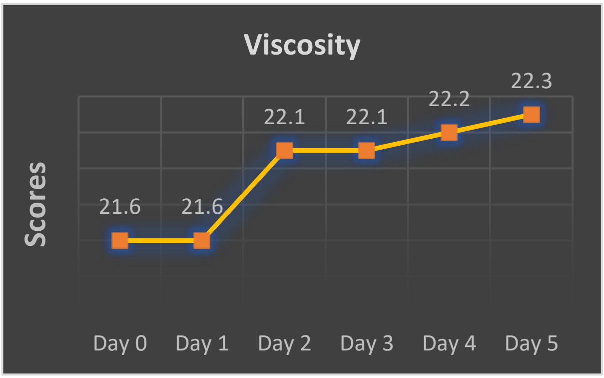


Fig 5.4 (d) Viscosity of fresh ambils

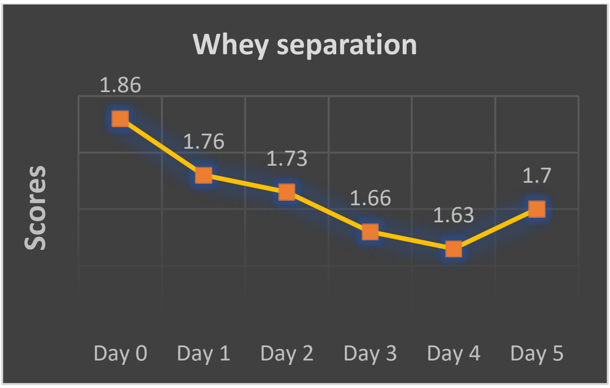


Fig 5.5 (e) Whey separation in fresh ambils

Fig 5.4 (a-e) Physicochemical properties of fresh Rice ambil over a storage period of 5 days

(c) Microbial parameters of freshly prepared rice ambils

Microbial studies for storage stability was carried for TPC, yeast and molds and coliform. It is evident from the following data (Table 5.24 and Fig 5.5) that all the microbiological quality attributes were significantly affected ($p<0.05$) upon storage period. Packaging in tight lid fitted glass bottles had not significant effect on microbiological quality of stored *ambil*. Total plate count on day zero was found to be 4.48 (log 10 cfu g^{-1}), which showed significant increase from day 1 (4.66 log 10 cfu g^{-1}) to day 5 (5.86 log 10 cfu g^{-1}) respectively. Yeast and mold count on day zero was found to be 2.63 (log 10 cfu g^{-1}) on day one, which upon storage in glass bottles at 4°C ±1 showed a significant increase ($p<0.05$) of 0.41% at day 5. Coliforms are an indicator of hygienic practices employed during product manufacture. It was found that coliforms were absent throughout the storage period in all the samples. Absence of coliform could be due to high heat treatment given to the cereals while preparation and subsequent hygienic handling of the product.

Table 5.24 Microbial changes in freshly prepared rice ambil over the storage period

Parameters / Days	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	p – value	%age difference
TPC (log 10 cfu g^{-1})	4.48 ^{a} ±0.03	4.66 ^{b} ±0.04	4.74 ^{b} ±0.02	5.24 ^{c} ±0.05	5.62 ^{d} ±0.02	5.86 ^{e} ±0.03	0.000	0.31
Yeast and mold (log 10 cfu g^{-1})	2.63 ^{a} ±0.05	2.79 ^{a} ±0.06	2.83 ^{a} ±0.13	2.92 ^{ac} ±0.07	3.28 ^{bc} ±0.12	3.72 ^{d} ±0.11	0.000	0.41

- Mean values represent the average of 10 determinants in triplicate
- a, b, c – The non-identical letters in any 2 columns within the row denote a significant difference at a minimum of 5% level
- NS – the difference between the mean values within the columns is not significant.
- Level of significance in increasing order – (*p<0.05, **p<0.01, ***p<0.001)

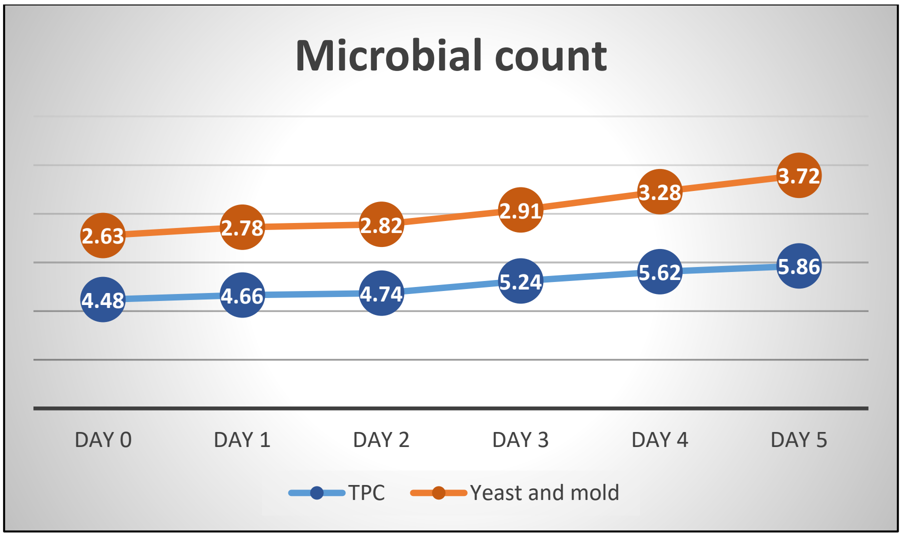


Fig 5.5 Microbial content of freshly prepared rice ambil over the storage period

5.2.6.2 Organoleptic evaluation, physicochemical properties and microbial content of barley ambils

(a) Organoleptic evaluation of freshly prepared barley ambil

Freshly prepared barley *ambil* was stored in refrigerator at 4°C ±1, there was a significant ($p<0.05$) difference in color and appearance during the storage period of 5 days, with a reduction of 13% from day 1 to day 5 (Table 5.25 and Fig 5.6 a-h). Consistency also changed significantly ($p<0.000$) during the storage period and decreased to 20% on day five. Aroma scores showed a significant difference ($p<0.000$) from day one (7.2) to the end of five days (5.5), likely due to increase in acidity. No significant difference in taste and flavour was observed up to 2 days, however after 2nd day there was slight reduction (31%) in the scores of taste and flavour. The reduction was significant from day 2 to day 5 ($p<0.000$), similarly no significant difference in mouthfeel was seen till day 2, but decreased after that (43%). Overall acceptability scores did not show any significant decrease till day 3 but gradually decreased by day 5, and the change was significant ($p<0.000$). Thus it can be concluded that overall no significant reduction in the scores till day 3 was observed.

Table 5.25 Mean scores for the sensory attributes of fresh Barley *ambil* over a storage period of 5 days

Parameters / Days	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	p – value	% age difference
Color	7.2^{a} ±0.61	7.1^{a} ±0.54	6.6^{b} ±0.67	6.4^{b} ±0.49	6.3^{b} ±0.46	6.3^{b} ±0.46	0.000	-13%
Consistency	7.1^{a} ±0.55	7^{b} ±0.45	6.2^{c} ±0.89	6^{c} ±0.79	5.7^{c} ±0.79	5.7^{c} ±0.79	0.000	-20%
Aroma	7.2^{a} ±0.61	6.9^{ab} ±0.71	6.6^{ac} ±0.67	6.2^{bcd} ±1.1	5.9^{cd} ±1.06	5.5^{d} ±1.3	0.000	-24%
Taste n flavour	7.2^{a} ±0.41	7^{a} ±0.64	6.3^{b} ±0.92	6^{b} ±1.02	5.1^{c} ±1.16	5^{c} ±1.2	0.000	-31%
Mouth feel	7.1^{a} ±0.55	6.9^{a} ±0.71	6.2^{b} ±0.76	5.8^{b} ±1.27	5^{c} ±0.91	4.7^{c} ±0.91	0.000	-43%
After taste	6.9^{a} ±0.71	6.8^{a} ±0.76	5.9^{b} ±1.06	5.6^{bc} ±1.04	5^{cd} ±0.64	4.6^{d} ±0.81	0.000	-17%
Absence of defects	6.9^{a} ±0.71	6.6^{a} ±0.81	5.7^{b} ±0.47	5.4^{bc} ±0.5	5^{cd} ±0.64	4.6^{d} ±1.13	0.000	-17%
OA	6.8^{a} ±0.76	6.7^{a} ±0.92	6.4^{a} ±1.04	5.9^{a} ±1.24	4.9^{b} ±1.06	4.6^{b} ±1.3	0.000	-33%

- Mean values represent the average of 10 determinants in triplicate
- a, b, c – The non-identical letters in any 2 columns within the row denote a significant difference at a minimum of 5% level
- NS – the difference between the mean values within the columns is not significant.
- Level of significance in increasing order – (*$p<0.05$, **$p<0.01$, ***$p<0.001$)

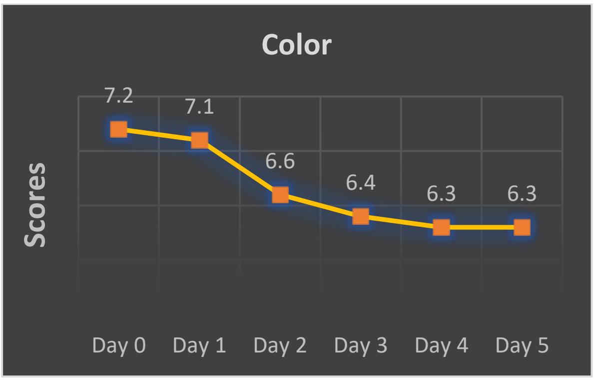


Fig 5.6 (a) Color of fresh ambils

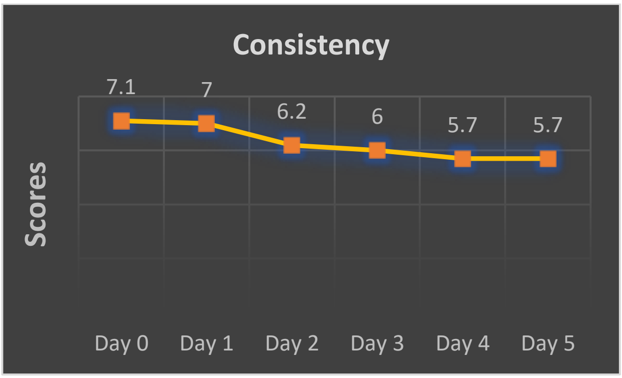


Fig 5.6 (b) Consistency of fresh ambils

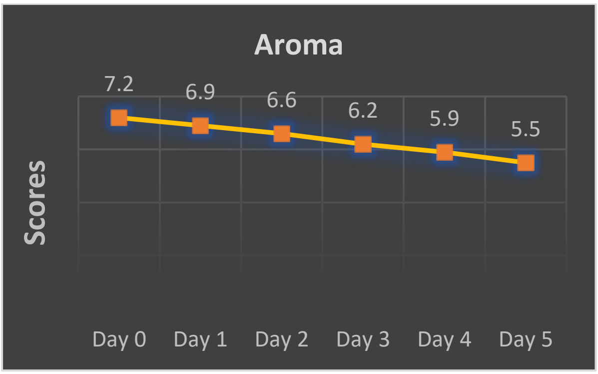


Fig 5.6 (c) Aroma of fresh ambils

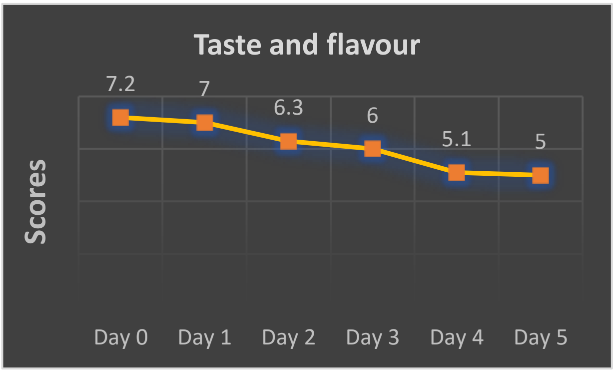


Fig 5.6 (d) Taste and flavour of fresh ambils

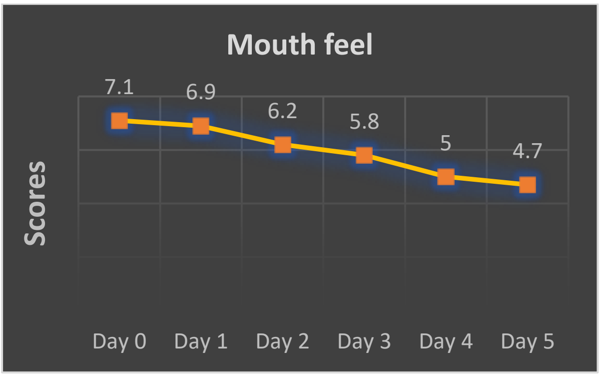


Fig 5.6 (e) Mouthfeel of fresh ambils

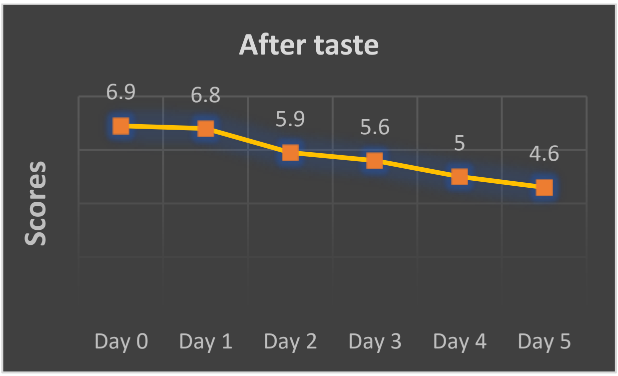


Fig 5.6 (f) After taste of fresh ambils

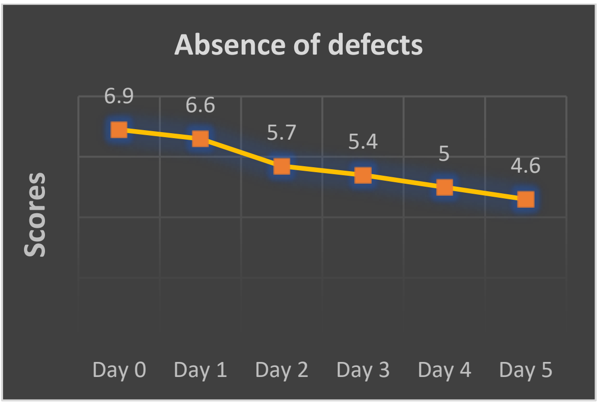


Fig 5.6 (g) Absence of defects in fresh ambils

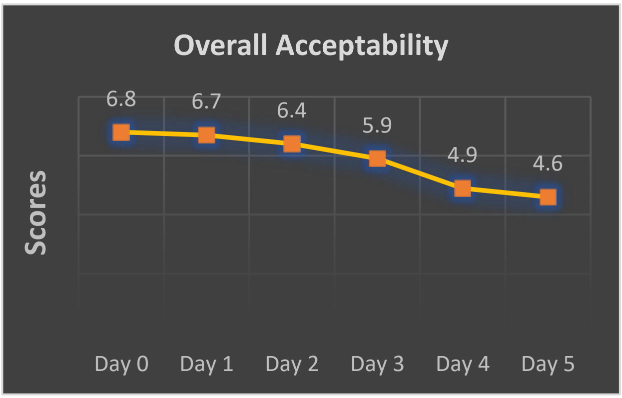


Fig 5.6 (h) Overall acceptabilty of fresh ambils

Fig 5.6 (a-h) Sensory attributes of fresh Barley ambil over a storage period of 5 days

(b) Physicochemical properties of freshly prepared barley ambil

A gradual decrease in pH and an increase acidity was observed during the storage. pH of freshly prepared barley *ambil* was significantly reduced by 0.29% from day 1 to day 5 (Table 5.26 and Fig 5.7 a-e). A significant increase in acidity was also observed over the storage period, ranging from 0.65 on day one to 1.12 on day five of storage (0.72% increase). No change in the peroxide value of the product was observed. Viscosity of barley *ambil* was found to be 22.7 (mPa.s @20°) at day 0, which increased by 0.02% at the end of day 5 (23.4 mPa.s @20°), however the increase was not significant. Whey separation index of different *ambils* was determined (expressed as ml whey expelled/ 10 ml of *ambil*). It gradually reduced by 0.21% over the storage period.

Table 5.26 Mean scores for the physicochemical properties of fresh Barley *ambil* over a storage period of 5 days

Parameters / Days	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	p – value	%age difference
pH	4.74 ^{a} ±0.02	4.68 ^{a} ±0.03	4.64 ^{ab} ±0.02	4.51 ^{b} ±0.01	4.16 ^{c} ±0.12	3.32 ^{d} ±0.05	0.000	-0.29%
Acidity	0.65 ^{a} ±0.01	0.72 ^{a} ±0.02	0.88 ^{bd} ±0.03	1.02 ^{b} ±0.35	1.06 ^{bc} ±0.05	1.12 ^{bc} ±0.01	0.000	0.72%
Peroxide value	<10	<10	<10	<10	<10	<10	-	-
Viscosity	22.7 ±0.26	22.9 ±0.21	22.9 ±0.30	22.9 ±0.05	22.8 ±0.60	23.4 ±0.20	0.151^{NS}	0.02%
Whey separation	1.88 ^{a} ±0.11	1.66 ^{b} ±0.01	1.60 ^{b} ±0.01	1.55 ^{b} ±0.01	1.53 ^{bd} ±0.07	1.47 ^{cd} ± 0.02	0.000	-0.21%

- Mean values represent the average of determinants in triplicate
- a, b, c – The non-identical letters in any 2 columns within the row denote a significant difference at a minimum of 5% level
- NS – the difference between the mean values within the columns is not significant.
- Level of significance in increasing order – (*p<0.05, **p<0.01, ***p<0.001)

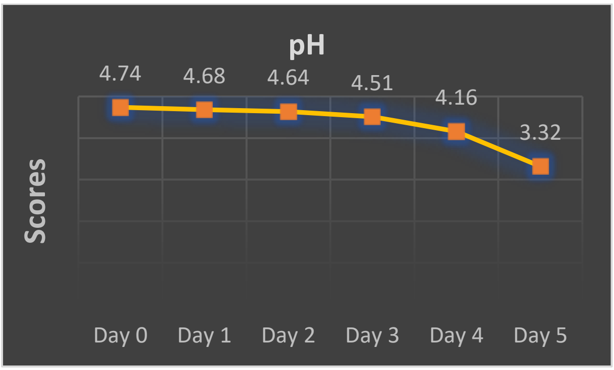


Fig 5.7 (a) pH of fresh ambils

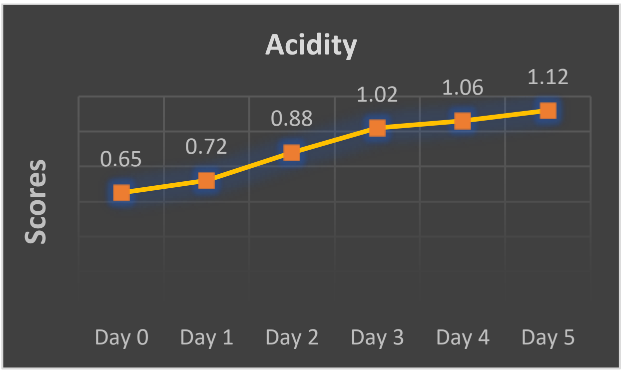


Fig 5.7 (b) Acidity in fresh ambils

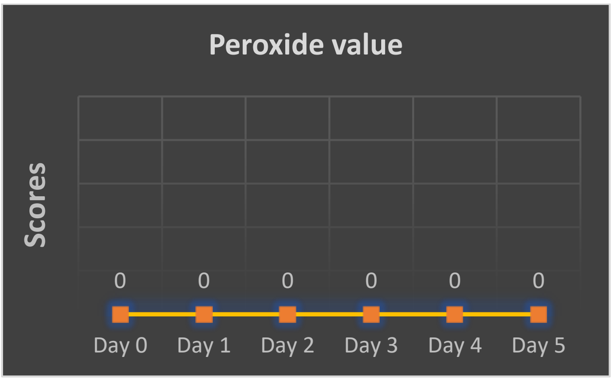


Fig 5.7 (c) Peroxide value of fresh ambils

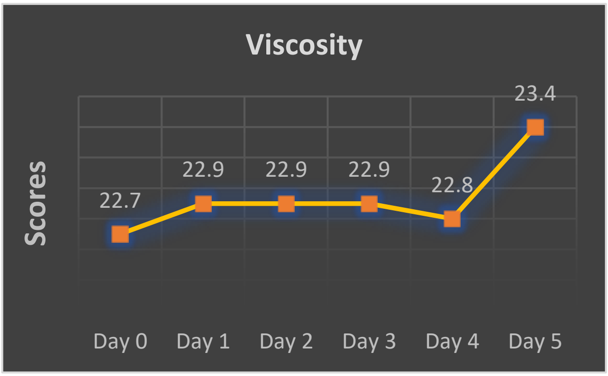


Fig 5.7 (d) Viscosity of fresh ambils

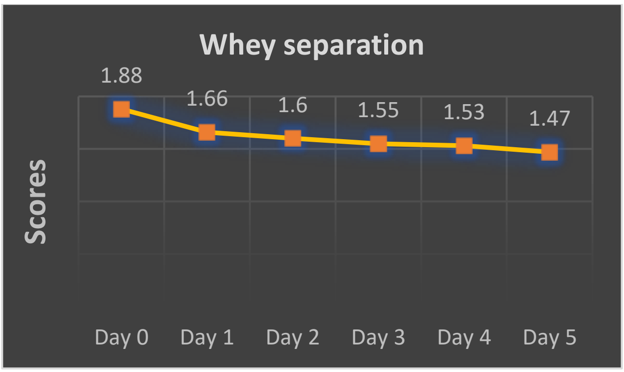


Fig 5.7 (e) Whey separation of fresh ambils

Fig 5.7 (a-e) Physicochemical properties of fresh Barley ambil over a storage period of 5 days

(c) Microbial parameters of freshly prepared barley ambil

Microbial studies for storage stability was carried for TPC, yeast and molds and coliform. It is evident from the following data (Table 5.27 and Fig 5.8) that all the microbiological quality attributes were significantly affected ($p<0.05$) upon storage period. Packaging in tight lid fitted glass bottles had not significant effect on microbiological quality of stored *ambil*. Total plate count on day zero was found to be 4.61 (log 10 cfu g^{-1}), which showed significant increase from day 1 (4.77 log 10 cfu g^{-1}) to day 5 (5.68 log 10 cfu g^{-1}) respectively. Yeast and mold count on day zero was found to be 2.54 (log cfu g^{-1}), which upon storage in glass bottles at 4°C ±1 showed a significant increase ($p<0.05$) of 0.27% at day 5. Coliforms are an indicator of hygienic practices employed during product manufacture. It was found that coliforms were absent throughout the storage period. Absence of coliform could be due to high heat treatment given to the cereals while preparation and subsequent hygienic handling of the product.

Table 5.27 Microbial changes in freshly prepared barley ambil over the storage period

Parameters / Days	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	p – value	%age difference
TPC (log 10 cfu g^{-1})	4.61 ^{a} ±0.02	4.77 ^{b} ±0.01	4.95 ^{c} ±0.05	5.21 ^{d} ±0.04	5.49 ^{e} ±0.03	5.69 ^{f} ±0.03	0.000	0.23
Yeast and mold (log 10 cfu g^{-1})	2.54 ^{a} ±0.06	2.67 ^{ab} ±0.08	2.79 ^{a} ±0.15	2.87 ^{ad} ±0.11	3.03 ^{bd} ±0.6	3.22 ^{cd} ±0.1	0.004	0.27

- Mean values represent the average of 10 determinants in triplicate
- a, b, c – The non-identical letters in any 2 columns within the row denote a significant difference at a minimum of 5% level
- NS – the difference between the mean values within the columns is not significant.
- Level of significance in increasing order – (*p<0.05, **p<0.01, ***p<0.001)

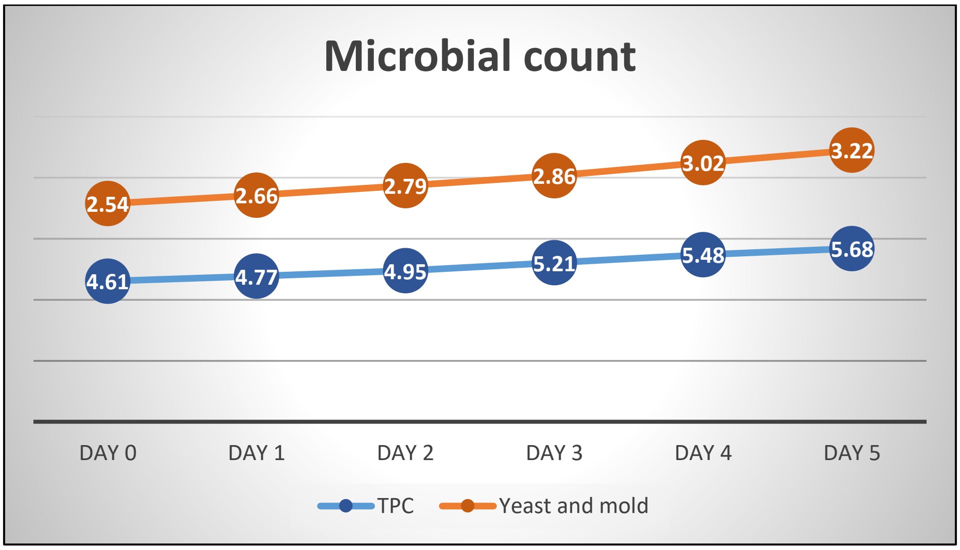


Fig 5.8 Microbial content of freshly prepared barley ambil over the storage period

5.2.6.3 Organoleptic evaluation, physicochemical properties and microbial content of pearl millet ambil

(a) Organoleptic evaluation of freshly prepared pearl millet ambil

Freshly prepared pearl millet *ambil* was stored in refrigerator at 4°C ±1, there was a significant ($p<0.05$) difference in color and appearance during the storage period of 5 days, with a reduction of 19% from day 1 to day 5 (Table 5.28 and Fig 5.9 a-h). Consistency also changed significantly ($p<0.000$) during the storage period and decreased to 13% on day five. Aroma scores showed a significant difference ($p<0.000$) from day one (7.6) to the end of five days (6.5), likely due to increase in acidity. No significant difference in taste and flavour was observed upto 2 days, however after 2nd day there was a reduction (38%) in the scores of taste and flavour.

The reduction was significant from day 3 to day 5 ($p<0.000$), similarly no significant difference in mouthfeel was seen till day 2, but decreased after that (42%). Overall acceptability scores did not show any significant decrease till day 2 but gradually decreased by day 5, and the change was significant ($p<0.000$) and the product was disliked by the panel members after day 4.

Table 5.28 Mean scores for the sensory attributes of fresh Pearl millet *ambil* over a storage period of 5 days

Parameters / Days	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	p – value	%age difference
Color	7.9^{a} ±0.71	7.8^{a} ±0.76	7.5^{ab} ±1.14	7^{bc} ±0.79	6.6^{cd} ±0.5	6.4^{d} ±0.67	0.000	-19%
Consistency	7.6^{a} ±1.04	7.5^{abd} ±1.04	7.1^{abc} ±0.84	6.9^{cd} ±0.84	6.6^{c} ±0.5	6.6^{c} ±0.49	0.000	-13%
Aroma	7.6^{a} ±0.67	7.2^{ab} ±6.41	7^{bc} ±0.64	6.7^{cd} ±0.47	6.5^{d} ±0.51	6.5^{d} ±0.50	0.000	-14%
Taste n flavour	7.1^{a} ±0.84	7^{a} ±0.79	6.5^{a} ±0.82	5.4^{b} ±0.81	4.7^{c} ±1.12	4.4^{c} ±1.03	0.000	-38%
Mouth feel	7.2^{a} ±1	6.8^{ab} ±0.61	6.3^{b} ±0.65	5.6^{c} ±0.81	4.5^{d} ±0.94	4.2^{d} ±0.76	0.000	-42%
After taste	6.8^{a} ±0.61	6.7^{a} ±0.47	6.1^{b} ±0.71	5^{c} ±0.91	4.6^{c} ±0.5	4.1^{d} ±0.30	0.000	-40%
Absence of defects	7.7^{a} ±0.79	7.3^{a} ±0.65	6.5^{b} ±0.68	5.9^{b} ±0.96	5.2^{c} ±0.89	4^{d} ±1.01	0.000	-29%
OA	7.1^{a} ±0.84	7^{ab} ±0.79	6.5^{b} ±0.51	5.8^{bd} ±0.41	4.6^{c} ±0.81	3.9^{c} ±1.06	0.000	-43%

- Mean values represent the average of 10 determinants in triplicate
- a, b, c – The non-identical letters in any 2 columns within the row denote a significant difference at a minimum of 5% level
- NS – the difference between the mean values within the columns is not significant.
- Level of significance in increasing order – (*$p<0.05$, **$p<0.01$, ***$p<0.001$)

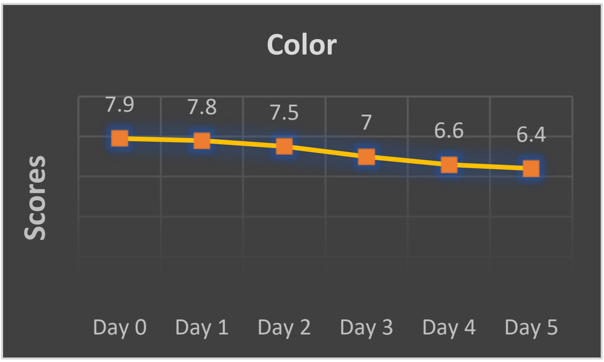


Fig 5.9 (a) Color of fresh ambils

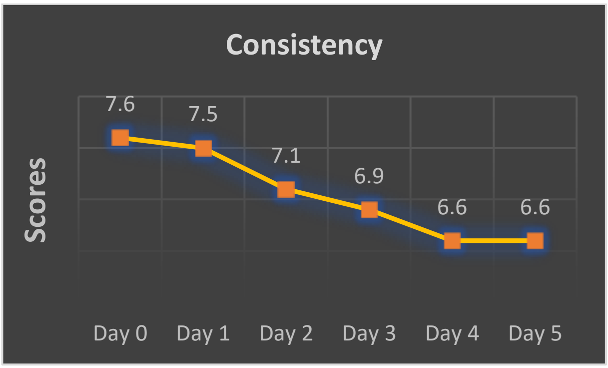


Fig 5.9 (b) Consistency of fresh ambils

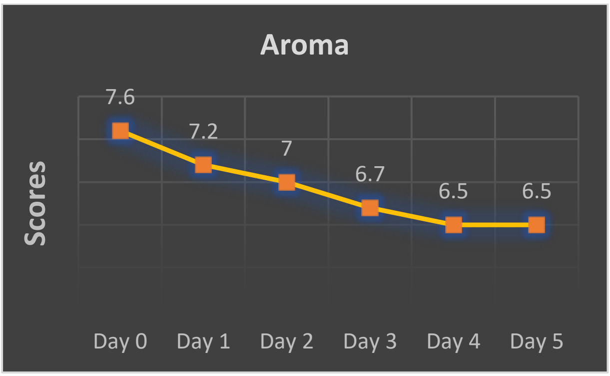


Fig 5.9 (c) Aroma of fresh ambils

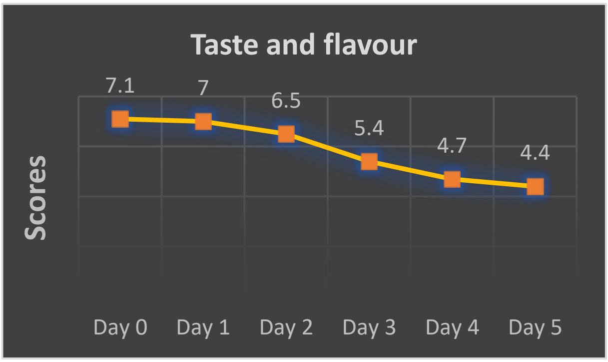


Fig 5.9 (d) Taste and flavour of fresh ambils

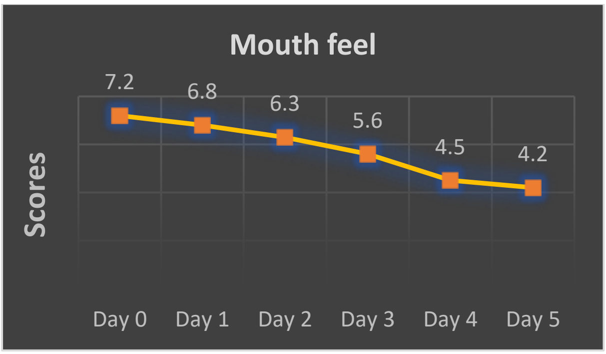


Fig 5.9 (e) Mouthfeel of fresh ambils

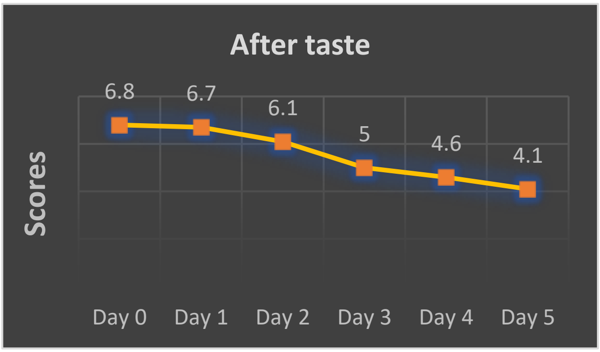


Fig 5.9 (f) After taste of fresh ambils

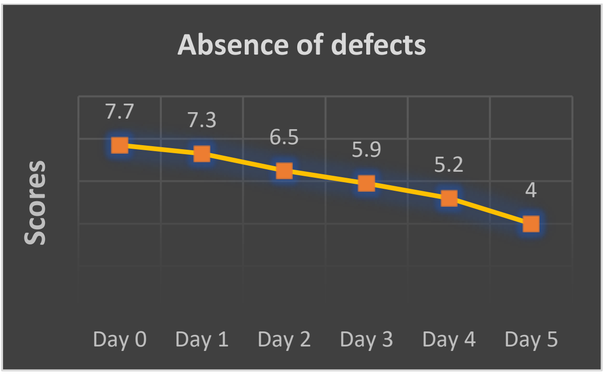


Fig 5.9 (g) Absence of defects fresh ambils

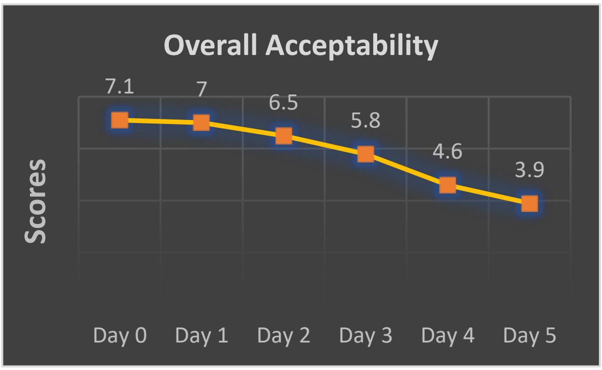


Fig 5.9 (h) Overall acceptability of fresh ambils

Fig 5.9 (a-h) Sensory attributes of fresh Pearl millet ambil over a storage period of 5 days

(b) Physicochemical parameters of freshly prepared pearl millet ambil

A gradual decrease in pH and an increase acidity was observed during the storage. pH of freshly prepared pearl millet *ambil* was significantly reduced by 0.23% from day 1 to day 5 (Table 5.29 and Table 5.10 a-e). A significant increase in acidity was also observed over the storage period, ranging from 0.45 on day one to 1.24 on day five of storage (1.77% increase). No change in the peroxide value of the product was observed. Viscosity of pearl millet *ambil* was found to be 21.5 (mPa.s @20°) at day 0, which increased by 0.04% at the end of day 5 (22.6 mPa.s @20°). Whey separation index of different *ambils* was determined (expressed as ml whey expelled/ 10 ml of *ambil*). It gradually reduced by 0.15% over the storage period.

Table 5.29 Mean scores for the physicochemical properties of fresh pearl millet *ambil* over a storage period of 5 days

Parameters / Days	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	p – value	%age difference
pH	4.7 [a] ±0.10	4.5 [b] ±0.02	4.4 [b] ±0.05	4.4 [b] ±0.04	3.90 [c] ±0.04	3.64 [c] ±0.04	0.000	-0.23%
Acidity	0.45 [a] ±0.01	0.55 [a] ±0.26	0.73 [ac] ±0.07	0.85 [a] ±0.06	1.12 [bc] ±0.13	1.24 [bd] ±0.01	0.000	1.77%
Peroxide value	<10	<10	<10	<10	<10	<10	-	-
Viscosity	21.5 [a] ±0.01	21.7 [ab] ± 0.2	21.9 [ab] ±0.15	22.1 [bc] ±0.05	22.4 [cd] ±0.2	22.6 [d] ±0.32	0.000	0.04%
Whey separation	2.23 [a] ±0.05	2.1 [a] ±0.1	1.94 [b] ±0.02	1.94 [b] ±0.02	1.87 [b] ±0.01	1.88 [b] ±0.01	0.000	-0.15%

- Mean values represent the average of determinants in triplicate
- a, b, c – The non-identical letters in any 2 columns within the row denote a significant difference at a minimum of 5% level
- NS – the difference between the mean values within the columns is not significant.
- Level of significance in increasing order – (*p<0.05, **p<0.01, ***p<0.001)

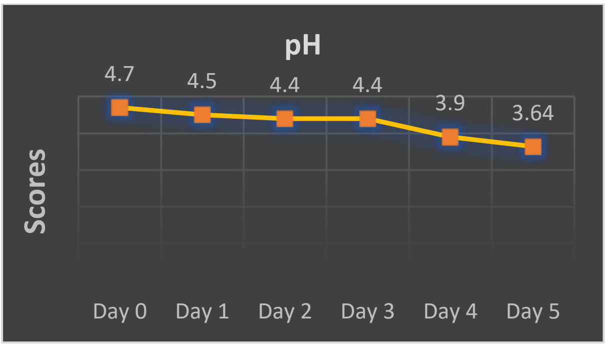


Fig 5.10 (a) pH of fresh ambils

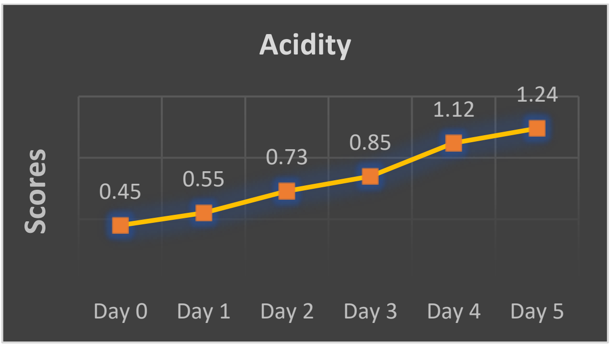


Fig 5.10 (b) Acidity of fresh ambils

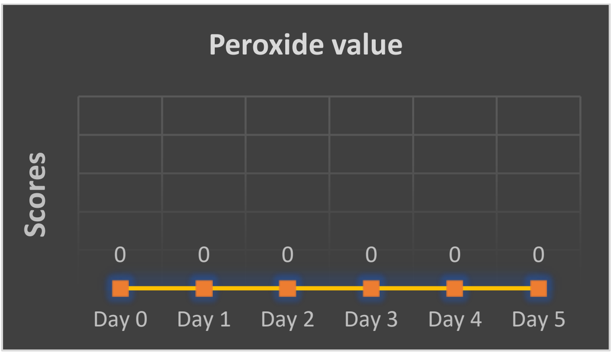


Fig 5.10 (c) Peroxide value of fresh ambils

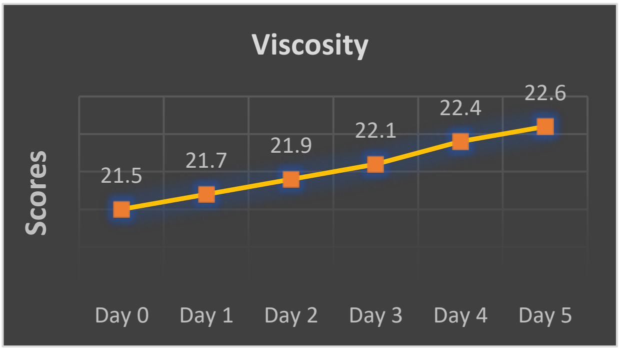


Fig 5.10 (d) Viscosity of fresh ambils

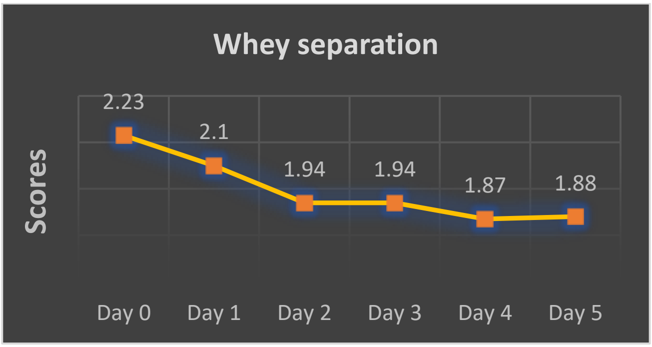


Fig 5.10 (e) Whey separation in fresh ambils

Fig 5.10 (a-e) Physicochemical properties of fresh pearl millet ambil over a storage period of 5 days

(c) Microbial parameters of freshly prepared pearl millet ambil

Microbial studies for storage stability was carried for TPC, yeast and molds and coliform. It is evident from the following data (Table 5.30 and Fig 5.11) that all the microbiological quality attributes were significantly affected ($p<0.05$) upon storage period. Packaging in tight lid fitted glass bottles had not significant effect on microbiological quality of stored *ambil*. Total plate count on day zero was found to be 4.42 (log 10 cfu g^{-1}),which showed significant increase from day 1(4.98 log 10 cfu g^{-1}) to day 5 (5.86 log 10 cfu g^{-1}) respectively. Yeast and mold count showed a significant difference from day zero 2.66 (log cfu g^{-1}), which upon storage in glass bottles at 4°C ±1 increased ($p<0.05$) by 0.38% on day 5. Coliforms are an indicator of hygienic practices employed during product manufacture. It was found that coliforms were absent throughout the storage period. Absence of coliform could be due to high heat treatment given to the cereals while preparation and subsequent hygienic handling of the product.

Table 5.30 Microbial content of freshly prepared pearl millet ambil over the storage period

Parameters / Days	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	p – value	%age difference
TPC (log 10 cfu g^{-1})	4.42 ^{a} ±0.04	4.98 ^{b} ±0.07	5.13 ^{bc} ±0.02	5.40 ^{c} ±0.07	5.75 ^{d} ±0.07	5.86 ^{d} ±0.1	0.000	0.33
Yeast and mold (log 10 cfu g^{-1})	2.66 ^{a} ±0.15	2.74 ^{a} ±0.16	2.86 ^{a} ±0.05	2.98 ^{ac} ±0.15	3.48 ^{c} ±0.10	3.68 ^{d} ±0.14	0.001	0.38

- Mean values represent the average of 10 determinants in triplicate
- a, b, c – The non-identical letters in any 2 columns within the row denote a significant difference at a minimum of 5% level
- NS – the difference between the mean values within the columns is not significant.
- Level of significance in increasing order – (*p<0.05, **p<0.01, ***p<0.001)

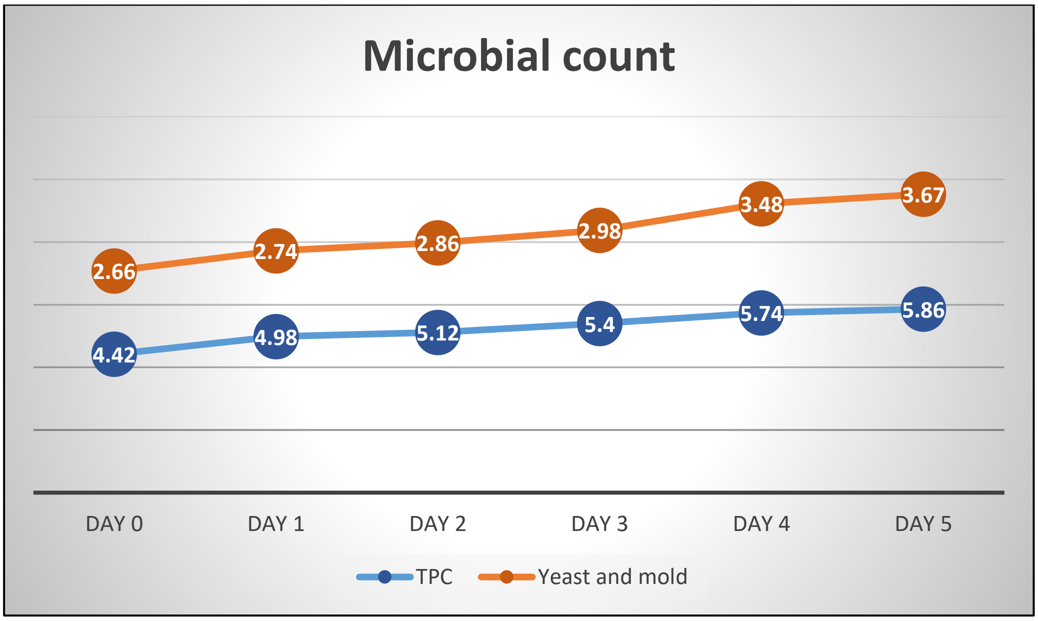


Fig 5.11 Microbial content of freshly prepared pearl millet ambil over the storage period

5.2.6.4 Organoleptic evaluation, physicochemical properties and microbial content of finger millet ambil

(a) Organoleptic evaluation of freshly prepared finger millet ambil

Freshly prepared finger millet *ambil* was stored in refrigerator at 4°C ±1, there was a significant ($p<0.05$) difference in color and appearance during the storage period of 5 days (Table 5.31 and Fig 5.12 a-h), with a reduction of 27% from day 1 to day 5. Consistency also changed significantly ($p<0.000$) during the storage period and decreased to 23% on day five. Aroma scores showed a significant difference ($p<0.000$) from day one (6.8) to the end of five days (3.9), likely due to increase in acidity. A significant reduction ($p<0.000$) in taste and flavour was observed after day 0 (58%), similarly a significant decrease in mouthfeel was observed after day 0 (48%). Overall acceptability scores did not show any significant decrease on day 1 but gradually decreased by day 5, and the change was significant ($p<0.000$). Thus it can be concluded that overall there was a significant reduction in the scores from day 1, also the product was graded as "highly disliked" at the day 5.

Table 5.31 Mean scores for the sensory attributes of fresh finger millet *ambil* over a storage period of 5 days

Parameters / Days	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	p – value	%age difference
Color	7 ^{a} ±1.44	6.8 ^{a} ±1.49	6.6 ^{a} ±1.59	5.8 ^{ab} ±2.12	5.3 ^{b} ±1.82	5.1 ^{b} ±1.53	0.000	-27%
Consistency	6.9^{a} ±0.55	6.8^{ab} ±0.41	6.3^{b} ±0.65	6.3^{b} ±0.65	5.6 ^{c} ±0.81	5.3^{c} ±1.02	0.000	-23%
Aroma	6.8 ^{a} ±0.61	6 ^{b} ±0.45	5.7^{bc} ±0.65	5.1^{c} ±0.84	4.4 ^{d} ±1.13	3.9^{d} ±1.39	0.000	-43%
Taste n flavour	6.6 ^{a} ±0.81	5.7^{b} ±0.79	4.9 ^{c} ±0.84	4.2 ^{d} ±0.41	3.2^{e} ±0.76	2.8^{e} ±0.76	0.000	-58%
Mouth feel	6.6^{a} ±0.5	5.4 ^{b} ±0.93	5.2^{b} ±0.89	4.3^{c} ±0.92	3.4^{d} ±0.93	3.4^{d} ±0.93	0.000	-48%
After taste	5.9 ^{a} ±1.16	5.4 ^{ab} ±0.93	4.8^{bc} ±0.89	4.6^{c} ±0.81	3.6 ^{d} ±1.04	3.5 ^{d} ±1.13	0.000	-29%
Absence of defects	6.5 ^{a} ±0.68	6 ^{a} ±0.64	5 ^{b} ±0.45	4.9 ^{b} ±0.31	4 ^{c} ±0.64	3.3 ^{d} ±1.20	0.000	-29%
OA	5.2 ^{a} ±0.89	4.9 ^{a} ±0.96	4.2 ^{b} ±0.76	3.8 ^{b} ±0.61	3.2 ^{bc} ±0.76	2.8 ^{c} ±0.88	0.000	-33%

- Mean values represent the average of 10 determinants in triplicate
- a, b, c – The non-identical letters in any 2 columns within the row denote a significant difference at a minimum of 5% level
- NS – the difference between the mean values within the columns is not significant.
- Level of significance in increasing order – (*$p<0.05$, **$p<0.01$, ***$p<0.001$)

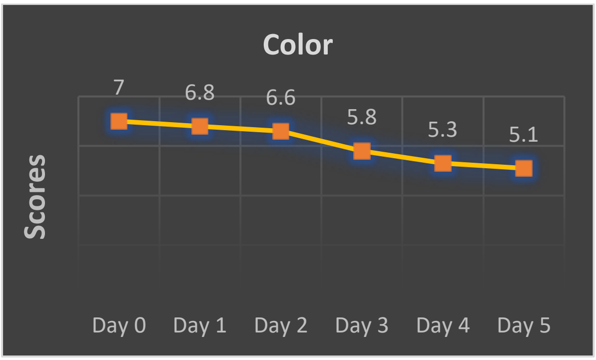


Fig 5.12 (a) Color of fresh ambils

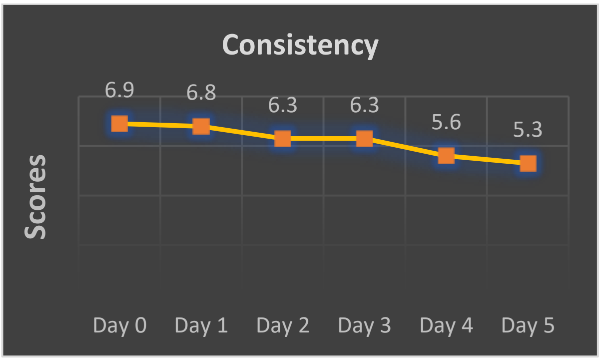


Fig 5.12 (b) Consistency of fresh ambils

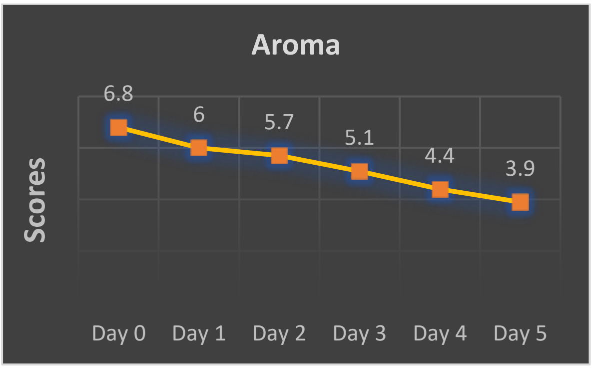


Fig 5.12 (c) Aroma of fresh ambils

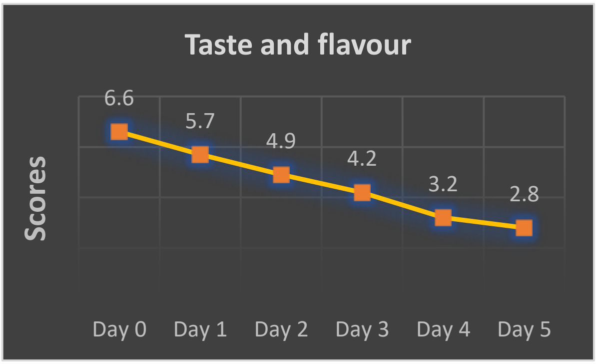


Fig 5.12 (d) Taste and flavour of fresh ambils

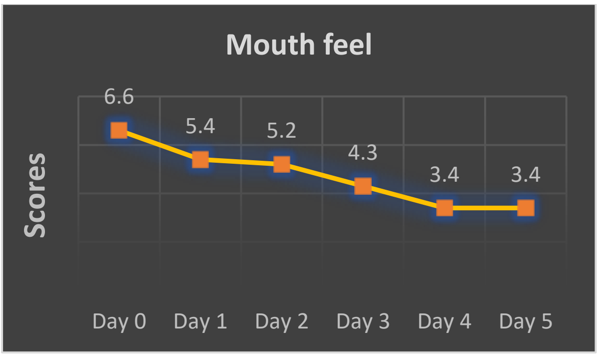


Fig 5.12 (e) Mouthfeel of fresh ambils

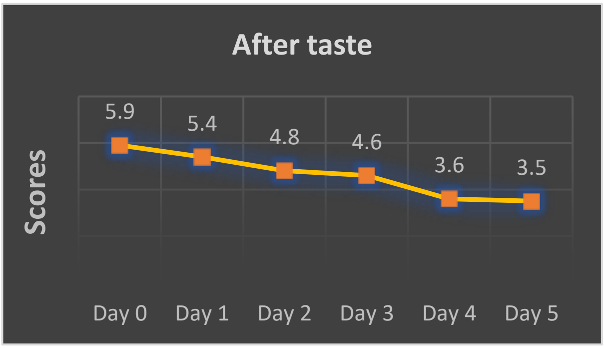


Fig 5.12 (f) After taste of fresh ambils

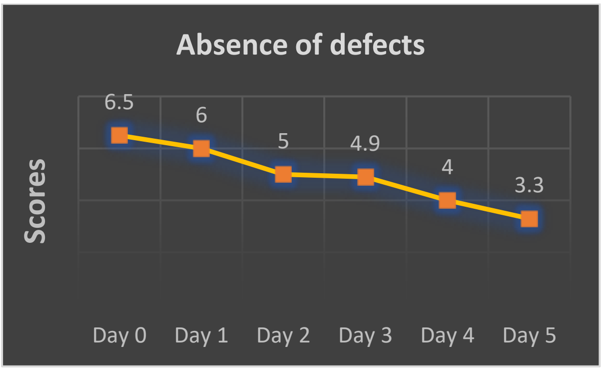


Fig 5.12 (g) Absence of defects in fresh ambils

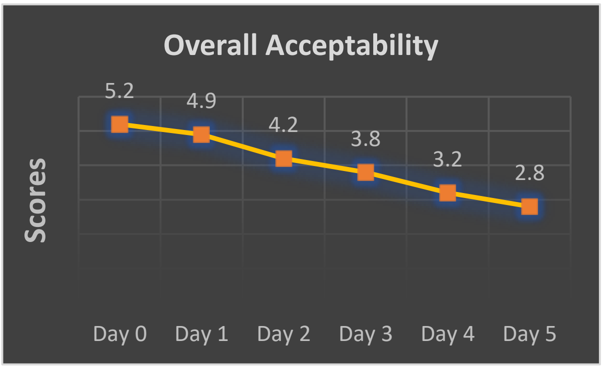


Fig 5.12 (h) Overall acceptability of fresh ambils

Fig 5.12 (a-h) Sensory attributes of fresh finger millet ambil over a storage period of 5 days

(b) Physicochemical parameters of freshly prepared finger millet ambil

A gradual decrease in pH and an increase acidity was observed during the storage. pH of freshly prepared finger millet *ambil* was significantly reduced by 0.10% from day 1 to day 5 (Table 5.32 and Fig 5.13 a-e). A significant increase in acidity was also observed over the storage period, ranging from 0.32 on day one to 1.61 on day five of storage (2.25% increase). No changes in the peroxide value of the product was observed. Viscosity of finger millet *ambil* was found to be 19.5 (mPa.s @20°) at day 0, which increased by 0.04% at the end of day 5 (20.3 mPa.s @20°). Whey separation index of different *ambils* was determined (expressed as ml whey expelled/ 10 ml of *ambil*). It gradually reduced by 0.20% over the storage period.

Table 5.32 Mean scores for the physicochemical properties of fresh finger millet *ambil* over a storage period of 5 days

Parameters / Days	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	p – value	%age difference
pH	3.97 [a] ±0.01	3.84 [b] ±0.02	3.82 [b] ±0.05	3.68 [c] ±0.01	3.60 [d] ±0.01	3.56 [d] ±0.02	0.000	-0.10%
Acidity	0.32 [a] ±0.05	0.43 [a] ±0.11	0.55 [b] ±0.08	0.95 [b] ±0.08	1.56 [c] ±0.04	1.61 [c] ±0.03	0.000	2.25%
Peroxide value	<10	<10	<10	<10	<10	<10	-	-
Viscosity	19.5 [a] ±0.15	19.5 [a] ±0.05	19.5 [a] ±0.04	19.8 [ab] ±0.12	20.1 [bd] ±0.05	20.3 [cd] ±0.15	0.000	0.04%
Whey separation	2.28 [a] ±0.03	2.17 [b] ±0.01	2.15 [bc] ±0.03	2.13 [bc] ±0.01	2.1 [ab] ±0.05	1.82 [d] ±0.02	0.000	-0.20%

- Mean values represent the average of determinants in triplicate
- a, b, c – The non-identical letters in any 2 columns within the row denote a significant difference at a minimum of 5% level
- NS – the difference between the mean values within the columns is not significant.
- Level of significance in increasing order – (*p<0.05, **p<0.01, ***p<0.001)

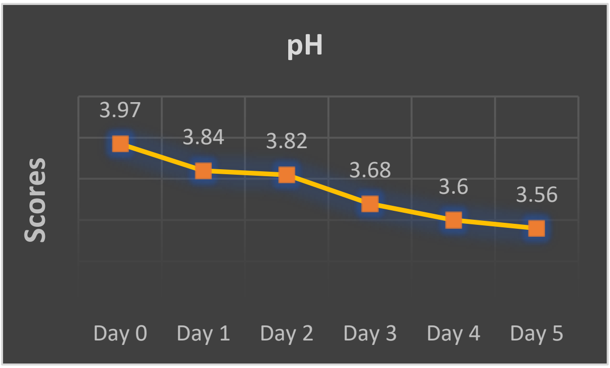


Fig 5.13 (a) pH of fresh ambils

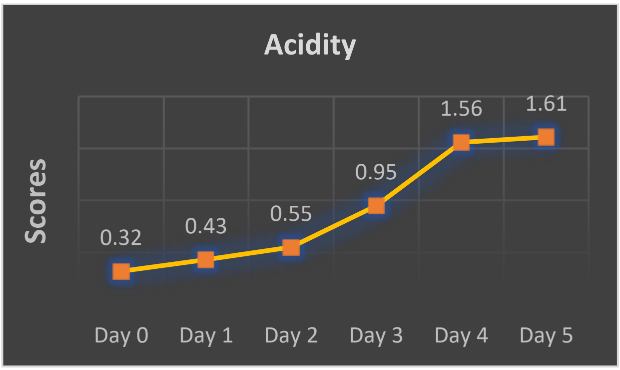


Fig 5.13 (b) Acidity of fresh ambils

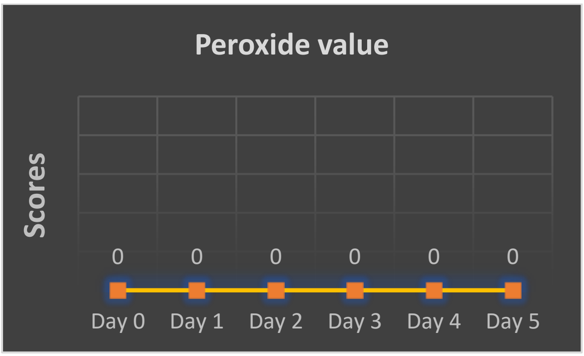


Fig 5.13 (c) Peroxide value of fresh ambils

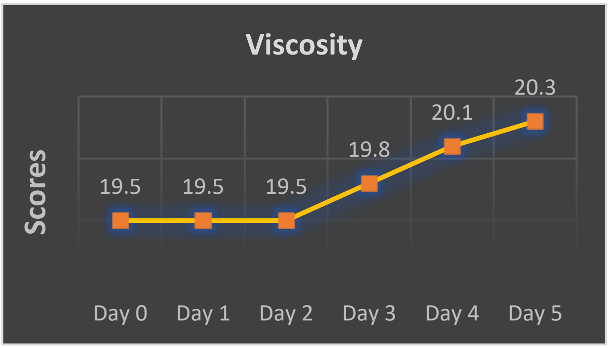


Fig 5.13 (d) Viscosity of fresh ambils

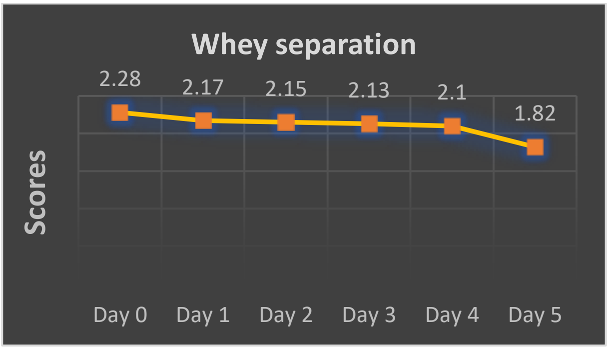


Fig 5.13 (e) Whey separation in fresh ambils

Fig 5.13 (a-e) Physicochemical properties of fresh finger millet ambil over a storage period of 5 days

(c) Microbial parameters of freshly prepared finger millet ambil

Microbial studies for storage stability was carried for TPC, yeast and molds and coliform. It is evident from the following data (Table 5.33 and Fig 5.14) that all the microbiological quality attributes were significantly affected ($p<0.05$) upon storage period. Packaging in tight lid fitted glass bottles had not significant effect on microbiological quality of stored *ambil*. Total plate count on day zero was found to be 4.96 (log 10 cfu g^{-1}), which showed significant increase from day 1 (5.24 log 10 cfu g^{-1}) to day 5 (6.24 log 10 cfu g^{-1}) respectively. Yeast and mold count on day zero was found to be 2.72 (log cfu), which upon storage in glass bottles at 4°C ±1 increased significantly by 0.52% at day 5. Coliforms are an indicator of hygienic practices employed during product manufacture. It was found that coliforms were absent throughout the storage period. Absence of coliform could be due to high heat treatment given to the cereals while preparation and subsequent hygienic handling of the product.

Table 5.33Microbial content of freshly prepared finger millet ambil over the storage period

Parameters / Days	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	p – value	%age difference
TPC (log 10 cfu g^{-1})	4.97 ^{a} ±0.09	5.25 ^{ac} ±0.12	5.46 ^{bc} ±0.11	5.74 ^{bd} ±0.11	6.02 ^{d} ±0.02	6.24 ^{d} ±0.11	0.000	0.26
Yeast and mold (log 10 cfu g^{-1})	2.72 ^{a} ±0.10	2.99 ^{a} ±0.07	3.37 ^{b} ±0.17	3.68 ^{bc} ±0.12	3.92 ^{c} ±0.07	4.13 ^{c} ±0.10	0.000	0.52

- Mean values represent the average of 10 determinants in triplicate
- a, b, c – The non-identical letters in any 2 columns within the row denote a significant difference at a minimum of 5% level
- NS – the difference between the mean values within the columns is not significant.
- Level of significance in increasing order – (*p<0.05, **p<0.01, ***p<0.001)

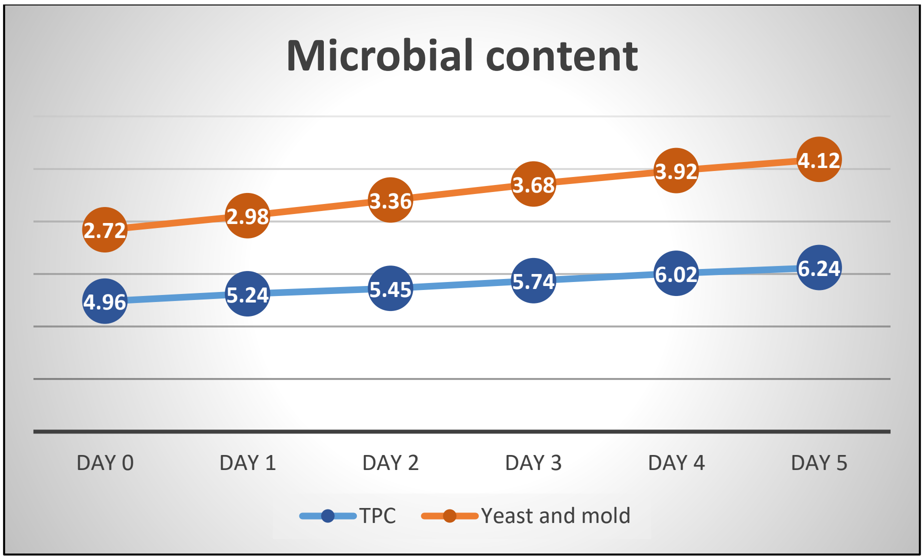


Fig 5.14 Microbial content of freshly prepared finger millet ambil over the storage period

5.2.7 Shelf life of packaged rice ambil

The packaged rice *ambil*, was stored at room temperature. The storage stability was studied for a period of 6 months including 0 day, 15th day, 30th day, 60th day, 3 months and 6 months. Organoleptic characteristics and physical and microbial parameters were studied including pH, acidity, viscosity, peroxide value, rancidity, TPC, coliform, yeast and mold.

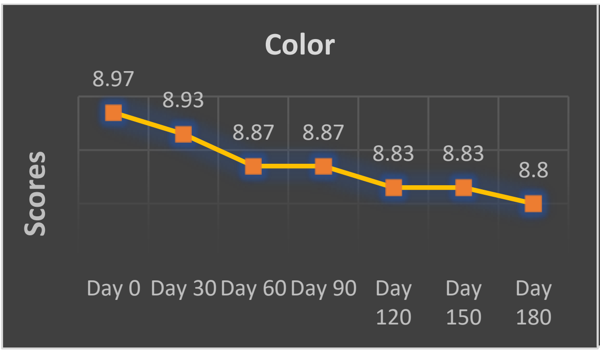
(a) ***Organoleptic evaluation of packaged rice ambil***

The storage stability was studied for a period of 6 months including 0 day, 15th day, 30th day, 60th day, 3 months and 6 months (Table 5.34 and Fig 5.15 a-h). There was no significant difference in color and appearance during the entire storage period. Consistency decreased by 3% during the storage period but the change was not significant. However, aroma scores showed a significant difference (p<0.000) from day zero to day 180, with a reduction of 4%. Results depicted in table 5.35 reveal that taste and flavour remain unaltered. Mouthfeel and after taste remained constant until 5 months and a slight decrease in the scores was seen at the end of 6th month. No change in the overall acceptability scores was observed throughout the storage period of 6 months. Thus it can be concluded that the acceptability of packaged rice *ambil* was till 6 months.

Table 5.34 Mean scores for the sensory attributes of packaged rice *ambil* over a storage period of 6 months

Parameters / Days	Day 0	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180	p-value	%age difference
Color	8.97 ±0.18	8.93 ±0.25	8.87 ±0.51	8.87 ±0.51	8.83 ±0.46	8.83 ±0.46	8.8 ± 0.29	0.822^{NS}	-0.01%
Consistency	8.93 ±0.25	8.9 ±0.4	8.57 ±0.51	8.77 ±0.73	8.77 ±0.73	8.66 ±0.71	8.66 ±0.7	0.455^{NS}	-0.03%
Aroma	9^{a} ±0.01	8.9^{a} ±0.31	8.9^{a} ±0.31	8.89^{a} ±0.21	8.8^{ab} ±0.41	8.6^{b} ±0.49	8.6^{b} ±0.5	0.000	-0.04%
Taste and flavour	9 ±0.02	8.97 ±0.18	8.93 ±0.25	8.9 ±0.31	8.9 ±0.3	8.8 ±0.46	8.8 ±0.41	0.155^{NS}	-0.02%
Mouth feel	8.97^{a} ±0.18	8.93^{a} ±0.25	8.9^{a} ±0.31	8.9^{a} ±0.25	8.9^{a} ±0.37	8.8^{a} ±0.46	8.5^{b} ±0.57	0.000	-0.05%
After taste	9^{a} ±0.01	8.9^{a} ±0.31	8.87^{a} ±0.38	8.87^{a} ±0.57	8.86^{a} ±0.35	8.86^{a} ±0.34	8.6^{b} ±0.05	0.006	-0.04%
Absence of defects	8.97^{a} ±0.18	8.93^{a} ±0.25	8.9^{a} ±0.4	8.9^{a} ±0.4	8.83^{b} ±0.53	8.76^{b} ±0.56	8.5^{b} ±0.63	0.001	-0.05%
OA	8.97 ±0.18	8.96 ±0.18	8.96 ±0.18	8.95 ±0.18	8.93 ±0.25	8.83 ±0.46	8.73 ±0.52	0.019^{NS}	-0.02%

Mean values represent the average of 10 determinants in triplicate
a, b, c – The non-identical letters in any 2 columns within the row denote a significant difference at a minimum of 5% level
NS – the difference between the mean values within the columns is not significant.
Level of significance in increasing order – (*p<0.05, **p<0.01, ***p<0.001)



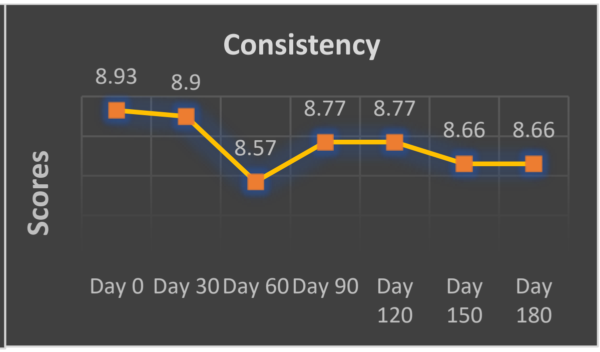
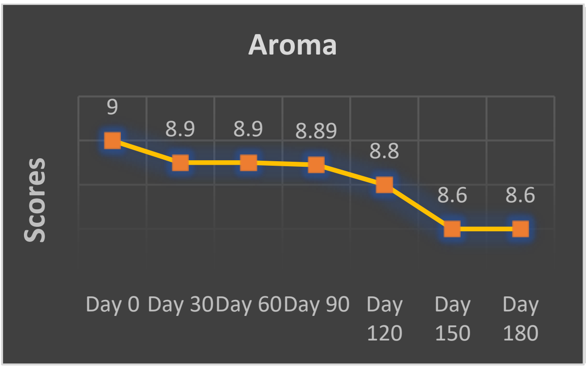


Fig 5.15 (a) Color of packaged rice ambil **Fig 5.15 (b) Consistency of packaged rice ambil**



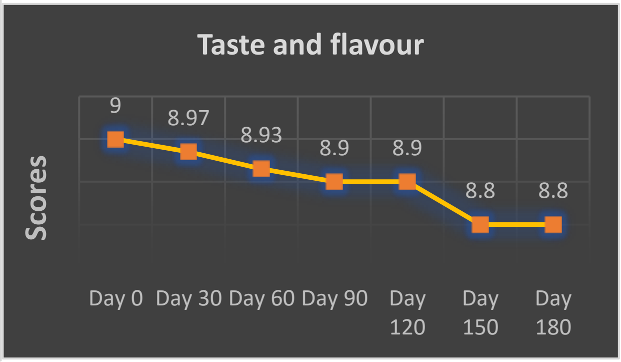
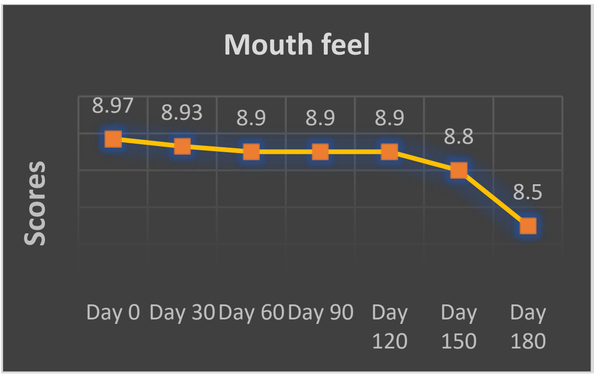


Fig 5.15 (c) Aroma of packaged rice ambil **Fig 5.15 (d) Taste and flavour of packaged rice ambil**



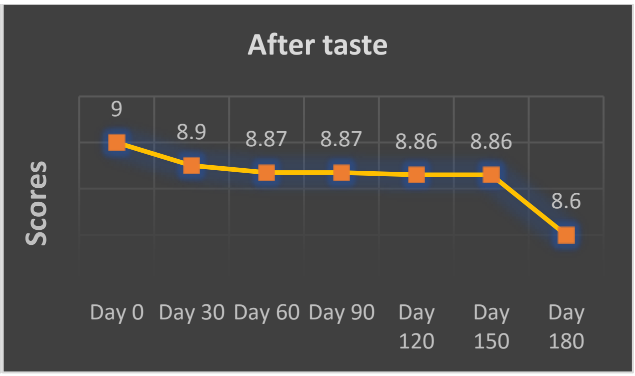
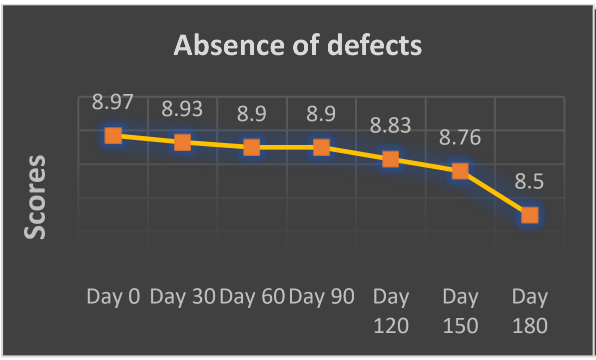


Fig 5.15 (e) Mouthfeel of packaged rice ambil **Fig 5.15 (f) After taste of packaged rice ambil**



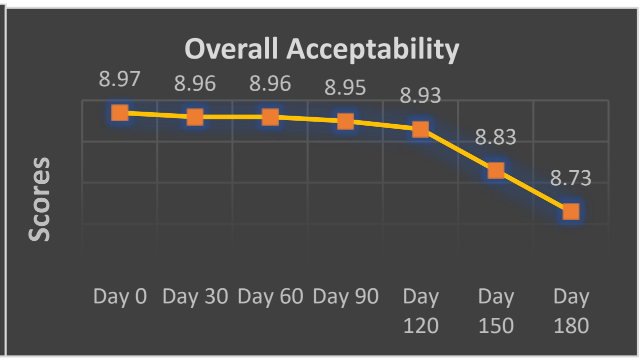


Fig 5.15 (g) Absence of defects in packaged rice ambil **Fig 5.15 (h) Overall acceptability of packaged rice ambil**

Fig 5.15 (a-h) Sensory attributes of packaged rice ambil over a storage period of 180 days (6 months)

(b) **Physicochemical properties of packaged rice ambil**

pH of packaged rice *ambil* on day 0 was found to be 4.6 and remained constant throughout the study period of 6 months (Table 5.35 and Fig 5.16 a-e), hence no significant difference. Acidity on day 0 was found to be 0.36 which was maintained throughout. Similarly no significant difference was seen in the viscosity, peroxide value and whey separation as they remained constant to 21.8 mPa.s @20°, peroxide value <10 meq/kg and whey separation 1.12 (expressed as ml whey expelled/ 10 ml of *ambil*) throughout the study period of 6 months.

Table 5.35 Mean scores for the physicochemical properties of packaged rice *ambil* over a storage period of 6 months

Parameters / Days	Day 0	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180	p-value	%age difference
pH	4.6 ±0.05	4.6 ±0.01	4.6 ±0.01	4.6 ±0.01	4.6 ±0.01	4.6 ±0.05	4.6 ±0.05	0.264[NS]	0
Acidity	0.36 ±0.01	0.36 ±0.05	0.36 ±0.00	0.36 ±0.01	0.36 ±0.01	0.36 ±0.01	0.36 ±0.05	0.932 [NS]	0
Peroxide value	<10	<10	<10	<10	<10	<10	<10	-	-
Viscosity	21.8 [a] ±0.05	21.8 [a] ±0.05	21.8 [a] ±0.1	21.8 [a] ±0.12	21.8 [a] ±0.11	22.3 [c] ±0.37	22.3 [c] ±0.11	0.001	0.02
Whey separation	1.12 ±0.02	1.12 ±0.00	1.12 ±0.00	1.12 ±0.01	1.12 ±0.02	1.13 ±0.05	1.14 ±0.02	0.471 [NS]	0.01

- Mean values represent the average of determinants in triplicate
- a, b, c – The non-identical letters in any 2 columns within the row denote a significant difference at a minimum of 5% level
- NS – the difference between the mean values within the columns is not significant.
- Level of significance in increasing order – (*p<0.05, **p<0.01, ***p<0.001)

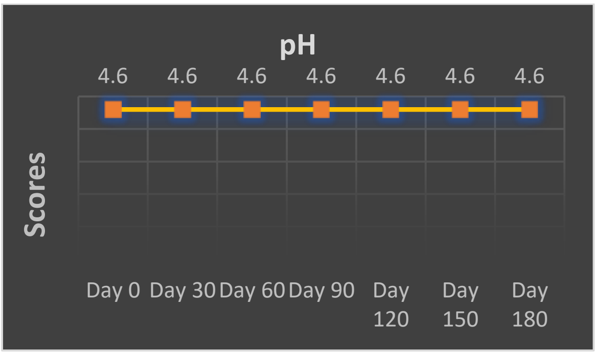


Fig 5.16 (a) pH of packaged rice ambil

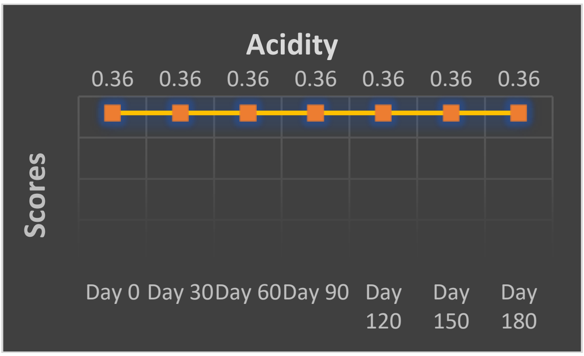


Fig 5.16 (b) Acidity of packaged rice ambil

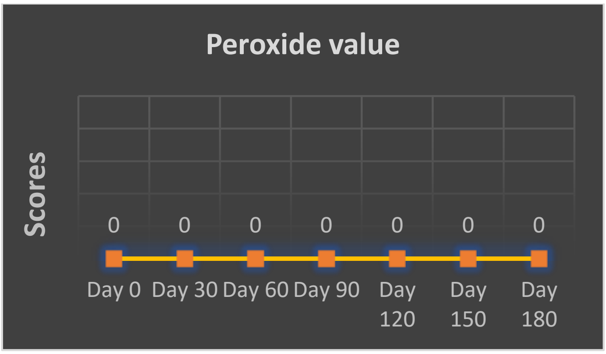


Fig 5.16 (c) Peroxide value of packaged rice ambil

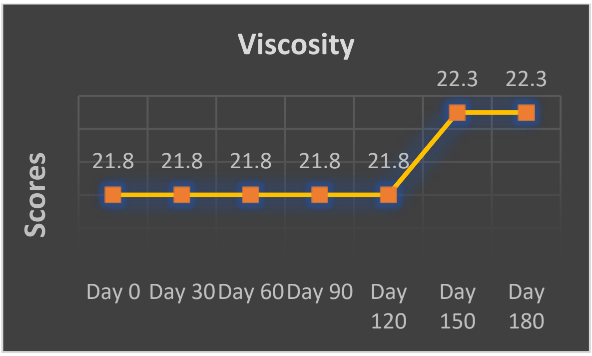


Fig 5.16 (d) Viscosity of packaged rice ambil

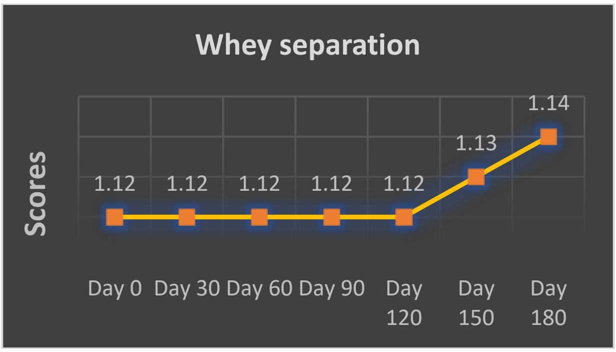


Fig 5.16 (e) Whey separation in packaged rice ambil

Fig 5.16 (a-e) Physical attributes of packaged rice ambil over a storage period of 180 days (6 months)

5.2.8 Summary of sensory and physical attributes of all fresh *ambils* at the end of 5 days

As seen in Table 5.36 it can be concluded that the taste and overall acceptability of rice ambil was scored best at the end of the storage period of 5 days (Table 5.36). Rice ambil had the highest acceptability at the end of day 5 followed by barley ambil and pearl millet ambil respectively. Finger millet ambil was least acceptable amongst all and was scored as "dislike moderately" at the end of day 5. A decrease in pH and increase in acidity was also observed amongst all the ambils, thus affecting the acceptability of ambils. Though barley ambil had the lowest pH at the end of day 5 but still was considered better as compared to pearl millet and finger millet ambil.

Table 5. 36 Summary of sensory and physical attributes of all fresh *ambils* at the end of 5 days

Ambils/ Parameters	pH	Acidity	Taste and flavour	Overall acceptability
Rice ambil	3.9 ±0.42	1.47 ±0.07	6 ±0.85	5.9 ±0.54
Barley ambil	3.32 ±0.05	1.12 ±0.01	5 ±1.2	4.6 ±1.3
Pearl millet ambil	3.64 ±0.04	1.24 ±0.01	4.4 ±1.03	3.9 ±1.06
Finger millet ambil	3.56 ±0.02	1.61 ±0.03	2.8 ±0.76	2.8 ±0.88

- Mean values represent the average of determinants in triplicate

Result highlights of Phase III

- Moisture (87.17%), pH (4.68) and acidity (0.56%) was highest in barley ambil as compared to other ambils.
- Significant difference in protein content was found between all the ambils, pearl millet ambil (1.22g) and barley ambil (1.22g) having the highest.
- Carbohydrate content and total calories was deterined, rice ambil had the highest CHO (6.4g) content and pearl millet ambil having highest calories (3.5kcal).
- Total fat (0.9g), saturated fat (0.2g) and MUFA (0.17g) content was highest in finger millet ambil.
- Folic acid and B vitamins were seen highest in pearl millet and barley ambils. Vitamin A content was highest in finger millet ambil (53 IU), and least in rice ambil (43 IU).
- Sodium (329 mg) and potassium (123mg) were found highest in barley ambil, magnesium (25.2mg) and phosphorus (35mg) highest in pearl millet ambils.
- Nutrient losses occurred during the UHT treatment in Vitamin B3 (-0.44%), folic acid (-0.04%), sodium (-0.27%) and potassium (-0.45%). Moisture content also reduced (-0.01%).
- Significant difference was observed in the shelf life of rice ambil (5.9) at the end of day 5, but still the product was graded fairly acceptable by the panel. Least acceptability was of finger millet ambil (2.8) at the end of day 5.
- Taste and flavour also degraded over the storage period of 5 days, least degradation was observed in rice ambil (6) followed by finger millet ambil (2.8).
- Microbial content also increased significantly over the period of 5 days but it was under the spoilage limits for all ambils.
- The storage stability of UHT packaged rice *ambil* was seen constant throughout the study period of 6 months and had shown no significant reduction in any of the storage parameters.

Discussion

Functional foods can be considered to be those whole, fortified, enriched or enhanced foods that provide health benefits beyond the provision of essential nutrients (e.g., vitamins and minerals), when they are consumed at efficacious levels as part of a varied diet on a regular basis. When food is being cooked or prepared using "scientific intelligence" with or without knowledge of how or why it is being used, the food is called "functional food". Thus, functional food provides the body with the required amount of vitamins, fats, proteins, carbohydrates, *etc*., needed for its healthy survival (FAO, 2016)

In recent years, cereals and its ingredients are accepted as functional food and nutraceuticals because of providing dietary fibre, proteins, energy, minerals, vitamins and antioxidants required for human health.

From a nutritional standpoint, dairy products are also a vital component of the food pyramid. This is mainly because they are very wholesome as they contain a wide variety of nutrients, and they are especially rich in proteins and calcium that is easily absorbed by our bodies.

Buttermilk is one of the most important functional dairy products that have excellent health and disease curing potentials and it is now receiving high interest from consumers all over the world. In addition, buttermilk is also considered as an excellent source of nutritional elements such as minerals (potassium, phosphorus, and calcium), vitamin B12, Vitamin B2, enzymes, and protein (Conway *et al*, 2014)

The nutritional analysis of buttermilk was determined, proteins, fats and carbohydrates were found to be 0.8g, 0.5gand 1.3g per 100ml respectively. Gebreselassie *et al*, 2016 also conducted chemical composition analysis of buttermilk and the fat content was found out to be 1.17 g per 100ml, similar results were also stated by Modha and Pal, 2011, stating the fat content in naturally fermented buttermilk was 1.2g per 100ml.

It is fundamental principle generally for food and specifically for milk that higher the temperature and shorter the processing time, the greater is the nutrient retention. Packaging of rice ambil involved UHT treatment, wherein it was expected that all the microbes will killed but some nutritional losses may have taken place. The results of the present study revealed that there was a reduction in moisture content after the treatment. These results agree with the moisture range found for UHT treated Brazilian bovine milks described by Do-Nascimento *et al*. (2010). In another study moisture content was evaluated before and after pasteurization and UHT treatment, which had shown a reduction from 88.65% to 88.22% (Pestana *et al*, 2015). In the current study the reduction in moisture content of ambils was found during the storage period which might be due to increase in total solids during storage. The present study can be supported by Datta *et al*, 2012 and Siddique *et al*, 2017 who described that when total solids increased ultimately moisture will be decreased and this happened more significantly in storage at higher temperature.

The ash content slightly increased (0.34%) as temperature was increased in the present study. The results supports the findings by Hussain (2011), who found that heating of skimmed milk samples resulted in increasing the ash content. These findings are in cross with the results by Siddiqui *et al*. (2010), stated that there was no significant difference in milk samples treated at various temperatures.

The results showed that fat content slightly increased after UHT treatment of ambils. These findings are in agreement to the results by Haq *et al* (2013) and Abubakar *et al*. (2001), who reported that increase in temperature, resulted in slight increase in fat content. This is further supported by Petrus *et al*. (2011) and Winarso *et al*. (2011), who reported that changes in chemical composition of fat could occur at higher temperatures under which a level of milk fat slightly reduced from that of fresh milk due to evaporation of some components of milk during heating process.

During milk processing significant losses of vitamin B3 have been reported upon sterilization and UHT treatment (30-35%) (Ritota, 2017). In another study conducted in Pakistan (Asadullah *et al*, 2010), milk samples collected from market were subjected to UHT treatment and nutritional losses were studied. Reduction in Vitamin B3 and folic acid were observed. In the present study as well slight decrease in Vitamin B3 and folic acid were observed, however no difference was found in Vitamin B1 and B2. Milk and other dairy products are important sources of vitamin B2. It is generally stable during the heat processing and normal cooking of foods, if light is excluded (Datta, 2018). However Vitamin B3 is unstable to high temperature (Kadakal *et al*, 2017; Moresch *et al*, 2009) and thus had showed reduction during the treatment.

Different technologically treated Italian milks (whole and semi-skimmed ultra-high temperature (UHT), pasteurized and microfiltered milk), collected from 2009 to 2012, were evaluated for nutritional and technological properties. No significant differences in calcium and sodium were detected ($p > 0.05$), while significant differences were observed concerning phosphorus content raw and UHT milk (Manzi *et al*, 2013). Similar results have been reported in the present study as well, with no significant change in sodium content upon the treatment, however a significant changes was observed in the calcium content.

Nutrient losses during the processing of UHT milk were studied, and results depicted that significant losses in folic acid, Vitamin B2, and Vitamin B1 were observed, however no significant losses were seen in Vitamin A, protein, mineral and trace elements (Riaz *et al*, 2009). In the present study as well no significant losses were seen in Vitamin A, likely because it is an oil soluble vitamin and is heat stable and thus is not adversely affected by sterilization or UHT process.

It is well known that, depending of numerous internal and external factors, the growth of microorganisms during the storage of dairy products results in their sensory changes, i.e. spoilage.

This is due to the fact that under the inadequate storage conditions, nutrients in the milk products are a good medium for the growth and development of individual groups of microorganisms (Tadeusz *et al*, 2005). The diverse range of dairy products found on the market today is a result of the knowledge and experience acquired over the years. In most cases, the microorganisms that cause the spoiling of milk and dairy products (Mostert and Jooste, 2002; Lafarge *et al*, 2004) reduce to some extent their quality. In the present study shelf life of fresh *ambils* and UHT treated packaged *ambils* were studied depicting various factors affecting the storage stability.

Shelf life of all the fresh *ambils* varied considerably throughout the period of 5 days. The average scores of colour, consistency, flavour, mouthfeel and overall acceptability of beverage on 5th day decreased for all the fresh *ambils*, and a drastic reduction of scores was seen in pearl millet and finger millet *ambil*. Overall acceptability of all the frrsh *ambils* reduced significantly from day 1 to day 5 which may be due to increase in acidity and decrease in pH of the *ambils* due to continued fermentation in the storage period because of the presence of micro organisms. The decrease in flavour scores may be mainly attributed to increase in acidity of beverage during storage period.

In a similar study, flavoured (strawberry, pineapple, and mango) probiotic acidophilus milk (from buffalo) using probiotic starter culture (Lactobacillus acidophilus) was prepared and its microbiological, physicochemical and sensory quality studies were carried out up to 6 days of storage. A slight increase in acidity of the milk was observed after 6 days of storage resulting in a decrease of pH (from pH 4.5 to 4.3). Sensory evaluation data shows that the quality of sensory attributes (color, taste, aroma, appearance and overall acceptability) decreased after 6 days of storage but still had considerable acceptability (Junaid *et al*, 2013).

Mugocha *et al*, 2000 had developed a fermented composite beverage using finger millet and milk for commercial production in Zimbabwe. A similar beverage called "*Raabdi*" was developed by Modha and Pal, 2011 using pearl millet, skim milk and buttermilk. The beverage was formulated and was stabilized using 0.6% pectin and CMC. The product was packaged in glass bottles and was stored under refrigeration (5-7 degree Celcius). The shelf life of the product was found to be 7 days, after which the product became unacceptable due to wheying-off and increase in acidity. Similar results for shelf life has been documented in the present study as well.

Khetarpaul *et al*, 1992 had prepared fermented drink using barley flour and sour buttermilk. The mixtures were fermented at different temperatures and timings varying from 30 to 40 degree Celsius for a period of 6 to 48 hours, shelf life of which was found to be 2 days, which coordinates with the results in the present study as well.

Storage of symbiotic *dahi* (Gawai and Shah, 2016) and synbiotic *lassi* (Sudheendra *et al*, 2018) was studied for a period of 7 to 14 days, depicting gradual decrease in the acceptability. Spoilage is mainly caused by excessive fermentation, enzymatic breakdown or contamination with undesirable microorganisms, all of which leads to increase in acidity and gas formation.

The analysis of variance showed that storage days significantly ($p<0.01$) affected the pH of *ambils*. The persistent growth and metabolic activity of lactic acid bacteria might have resulted in accumulation of organic acids and causes reduction in pH of fermented milks (Ruggeri *et al*., 2008). Presence of prebiotic polysaccharides and other growth promoting substances might have sustained the metabolic activity of lactic acid bacteria leading to decrease in pH of *ambils*. Hussain *et al*, 2015 had also documented similar reduction in pH upon the storage period of curd supplemented with aloevera, with a decrease from 3.99 to 3.45 from 0 to 12th day of storage.

Viscosity of fresh *ambils* were found to be 21.6, 21.5, 22.7 and 19.5 for rice, pearl millet, barley and finger millet *ambil* respectively which gradually increased to fifth day of storage. This slight increase in viscosity may be due gelatinization of starch (Masakuni *et al*. 2014) and interaction of protein with pectin having negative charge.

The wheying-off scores on 0 day were 1.86, 2.23, 1.88 and 2.28 for rice, pearl millet, barley and finger millet *ambil* respectively. The wheying-off scores on 5th day were 1.7, 1.8, 1.47 and 1.82 for rice, pearl millet, barley and finger millet *ambil* respectively. Analysis of variance showed that wheying-off scores differed significantly ($p<0.01$) between storage days. The decrease in wheying-off score could be ascribed to the persistent metabolic activity of starter culture which might have destabilized the whey protein and casein networks leading to oozing out of water (Sadeghi *et al*, 2012).

Significant difference was found in all the freshly prepared ambils throughout the storage period. Maximum amount of microbial growth was seen in finger millet ambil with an increase in TPC count from 4.96 to 4.64 from day zero to day 5^{th} of storage. The acidity of finger millet ambil was found maximum as compared to other ambils on day five, this may be due to high rate of fermentation by finger millet as compared to other cereals. These results were similar to a study conducted on preparation of south Indian foods *idli* and *dosa* using finger millet, which documented that the fermentation rate of finger millet flour was better than rice flour (Krishnamoorthy *et al*, 2013). The stoichiometry of fermentation was investigated in vitro with wheat and maize grains. Gas production proved to be an accurate index of VFA production and change in pH. There were significant differences between grain species in rates of gas production ($P < 0.001$), being ranked in the order wheat > triticale, oats > barley > maize > rice, sorghum (Liptáková *et al* 2016).

Ultra-high temperature processing (UHT), ultra-heat treatment, or ultra-pasteurization is a food processing technology that sterilizes liquid food, chiefly milk, by heating it above 135 °C (275 °F) – the temperature required to kill spores in milk – for 1 to 2 seconds (Chavan *et al*, 2011). UHT is most commonly used in milk production, but the process is also used for fruit juices, cream, soy milk, yogurt, wine, soups, honey, and stews (Deeth, 2018).

The storage stability of UHT packaged rice *ambil* was seen constant throughout the study period of 6 months and had shown no significant reduction in any of the storage parameters. Ultra High Temperature-treated *ambil* was heated to very high temperature (135 to 137 degree Celcius) for a brief period. It was been passed through heating and cooling stages in quick successions. Thus leading to a better shelf life stability and thus it was acceptable by the panel members up to 6 months.

Conclusion

- *Packaged rice ambil is a low calorie and low fat beverage with moderate calcium.*
- *Negligible nutrient losses occurred during UHT treatment of rice ambil*
- *Shelf life of all refrigerated fresh ambils was 3 days in terms of taste and overall acceptability. Shelf life of packaged ambil was upto 6 months.*
- *Finger millet ambil stayed good for 2 days and gradually the acceptability scores reduced significantly after that.*
- *Microbial content also increased significantly over the period of 5 days but it was under the spoilage limits for all ambils.*

Phase IV - Determination of Consumer acceptability and market potential of the most acceptable packaged rice *Ambil*

Consumer is broadly any individual that uses goods and services produced within the market. The concept of a consumer arises in diverse framework, so that the usage and significance of the term may differ (Kotler and Keller, 2011). Consumer buying behavior is the study of the ways of buying and disposing of goods, services, ideas or experiences by the individuals, groups and organizations in order to satisfy their needs and wants. Many of the health drinks available in the Indian market have their origins in traditional Ayurveda. Hence this traditional base has been taken as the basis of segregating health drinks. In this phase of the study an attempt was made to determine the frequency of consumption of health beverages and consumer acceptability of packaged rice ambil and its market potential. The market potential was studied in terms of idea and need of the product, expected cost, desirable flavours and package size of packaged rice ambil.

The results of this phase are presented in following sections:

5.3.1 Frequency of consumption of curd/ buttermilk by consumers

5.3.2 Frequency of consumption of fruit juices by consumers

5.3.3 Frequency of consumption of probiotic beverages by consumers

5.3.4 Consumer acceptability for packaged rice ambil

5.3.5 Market potential for packaged rice ambil

5.3.5.1 Consumer views on the idea of the packaged rice ambil

5.3.5.2 Consumer views on need of the packaged rice ambil commercially

5.3.5.3 Consumer preference for purchase of the packaged rice ambil

5.3.5.4 Consumer preference for cost of the packaged rice ambil

5.3.5.5 Consumer preference to recommend the packaged rice ambil to others

5.3.5.6 Consumer preference for flavours of the packaged rice ambil

5.3.5.7 Consumer preference for package size of the packaged rice ambil

5.3.1 Frequency of consumption of curd/ buttermilk by consumers

As can be seen in Table 5.37 and Fig 5.17, a significant difference (p<0.001) was found in the frequency of consumption of curd/ buttermilk amongst all the age groups. Majority of the subjects consumed buttermilk or curd on daily basis or more than once a week with highest (95.2%) consumption in the age group of 40-59 years and least (76.1%) in above 60 years of age.

Table 5.37 Frequency of consumption of curd/ buttermilk (Chi Square test)

Age Group	Daily	Once a month	Twice a month	Never	Chi square	p-value
10-19 (n=95)	80 (84.2)	8 (8.4)	4 (4.2)	3 (3.1)	21.49	0.000
20-39 (n= 186)	164 (88.1)	10 (5.3)	1 (1)	11 (5.9)		
40-59 (n=106)	101 (95.2)	2 (1.8)	0 (0)	3 (2.8)		
>60 **(n=42)**	32 (76.1)	9 (21.4)	0 (0)	1 (2.3)		

Note: Figures in parenthesis indicate percentages

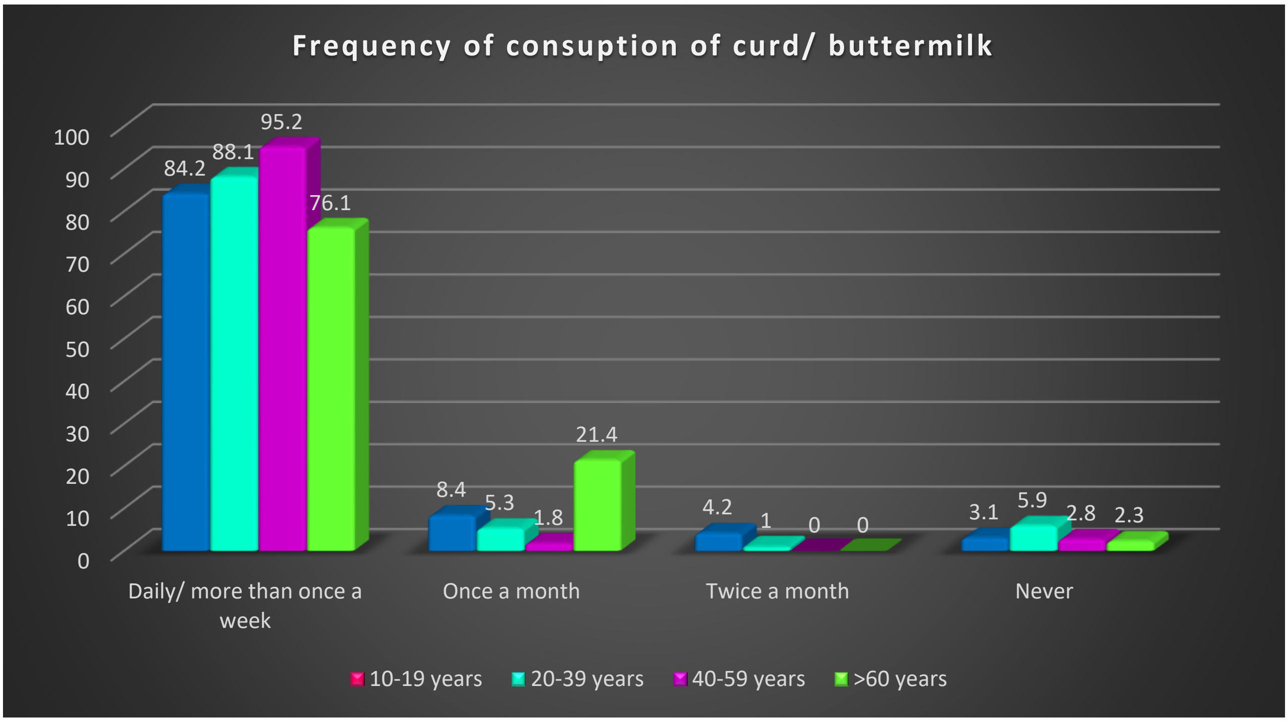


Fig 5.17 Frequency of consumption of curd/buttermilk (percentages)

5.3.2 Frequency of consumption of fruit juices by consumers

As can be seen in Table 5.38 and Fig 5.18, a significant difference ($p<0.001$) was found in the frequency of consumption of fruit juices amongst all the age groups. Consumption of fruit juices was found highest in the age group of 40-59 years (93.3%) followed by the age group of 20-39 years (89.7%). Daily consumption was found least (45.2%) in elderlies and 30.9% of the consumers stated that they never consume fruit juices.

Table 5.38 Frequency of consumption of fruit juice (Chi Square test)

Age Group	Daily	Once a month	Twice a month	Never	Chi Square	p-value
10-19 (n=95)	80 (84.2)	9 (9.4)	1 (1)	1 (1)	80.38	0.000
20-39 (n= 186)	167 (89.7)	13 (6.9)	13 (6.9)	6 (3.2)		
40-59 (n=106)	99 (93.3)	3 (2.8)	1 (0.9)	3 (2.8)		
>60 **(n=42)**	19 (45.2)	2 (4.7)	8 (19)	13 (30.9)		

Note: Figures in parenthesis indicate percentages

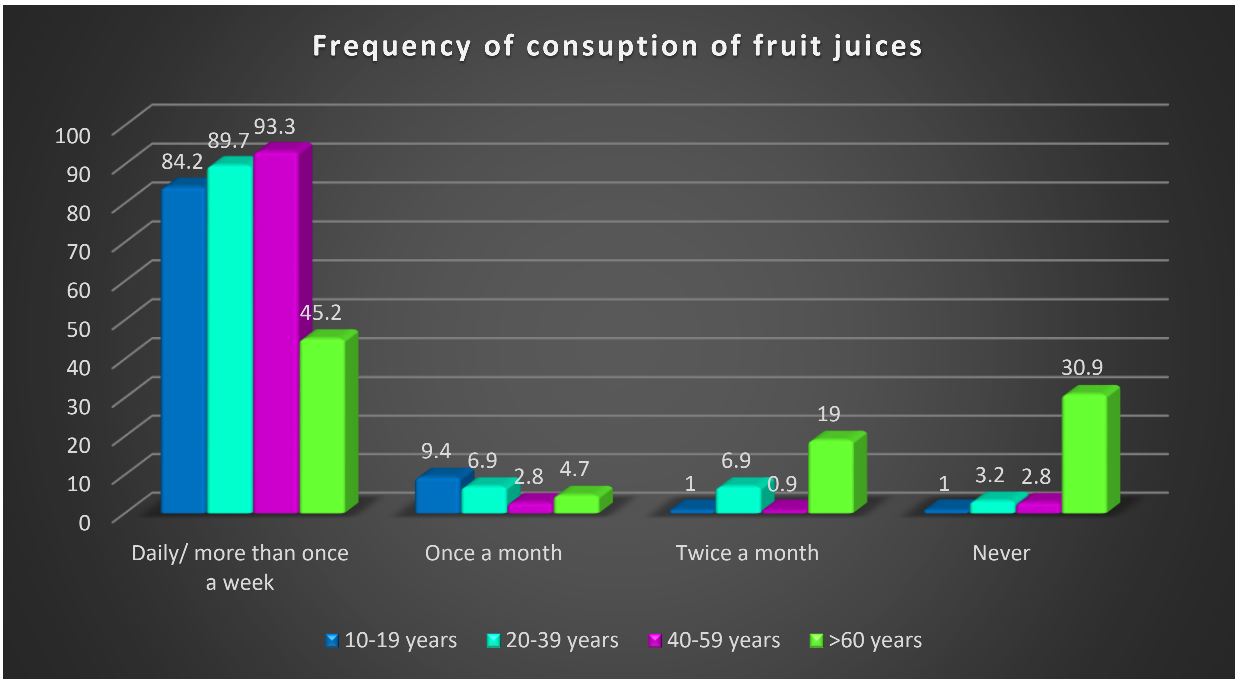


Fig 5.18 Frequency of consumption fruit juices (percentages)

5.3.3 Frequency of consumption of probiotic beverages by consumers

As can be seen in Table 5.39 and Fig 5.19, a significant difference ($p<0.001$) was found in the frequency of consumption of probiotic beverages amongst all the age groups. Daily consumption and more than once a week was found highest in the age group of 10-19 years (52.6%) followed by 20-39 years (37%). Fifty seven percent from the age group stated they never consume probiotic drinks followed by elderlies. Overall it was seen that out of all the subjects 37.7% consumed probiotic beverages either on daily basis or more than once a week.

Table 5.39 Frequency of consumption of probiotic drinks (Chi Square test)

Age Group	Daily	Once a month	Twice a month	Never	Chi Square	p-value
10-19 (n=95)	50 (52.6)	16 (16.8)	6 (6.3)	17 (17.8)	30.67	0.000
20-39 (n= 186)	69 (37)	38 (20.4)	10 (5.3)	69 (37)		
40-59 (n=106)	31 (29.2)	14 (13.2)	4 (3.7)	57 (53.7)		
>60 (n=42)	12 (28.5)	6 (14.2)	3 (7.1)	21 (50)		

Note: Figures in parenthesis indicate percentage

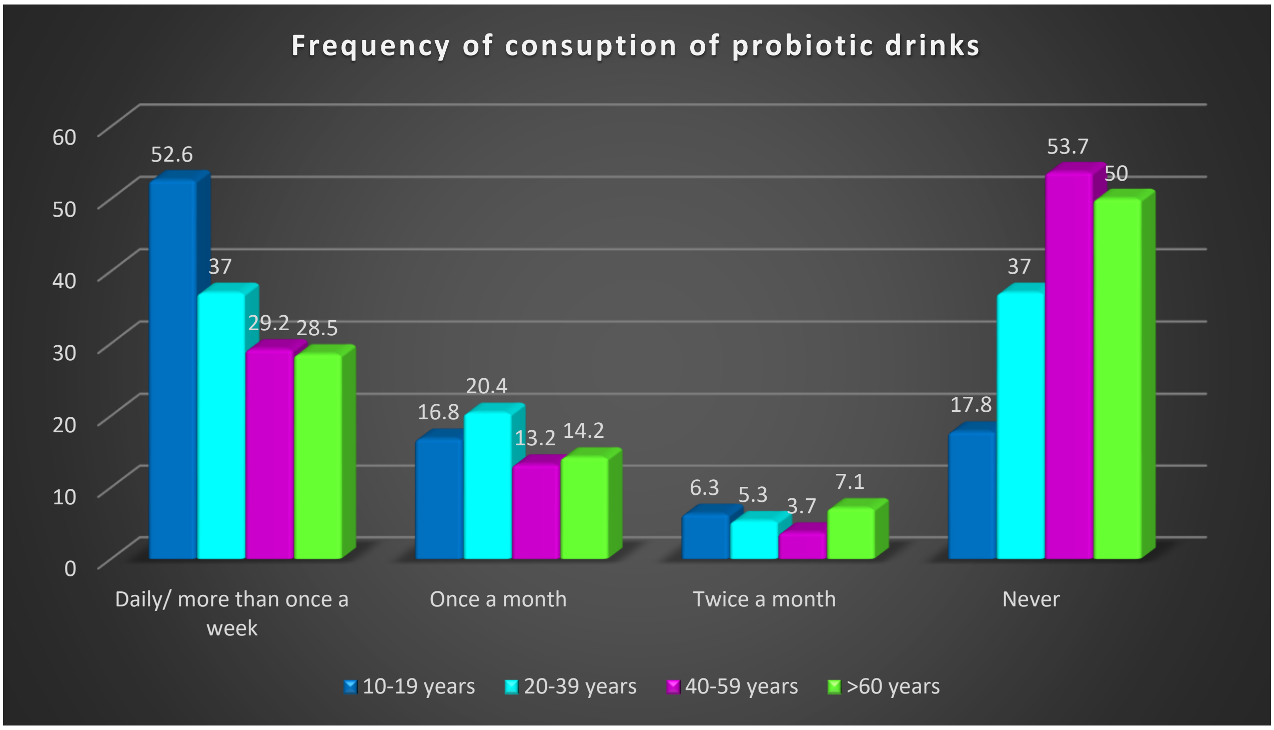


Fig 5.19 Frequency of consumption probiotic drinks (percentage)

5.3.4 Consumer acceptability of packaged rice ambil

A total of 518 subjects were selected using the percentage census of population. As can be seen in Fig 5.20 and Fig 5.21, out of all the subjects 303 were female and 215 male with 35.3 % less than 20 years of age, 56.7% were in the age range of 20- 59 yrs and remaining 7% were above 60 years of age. All the consumers were given packaged rice *ambil* to taste and were given a hedonic rating scale to score the product.

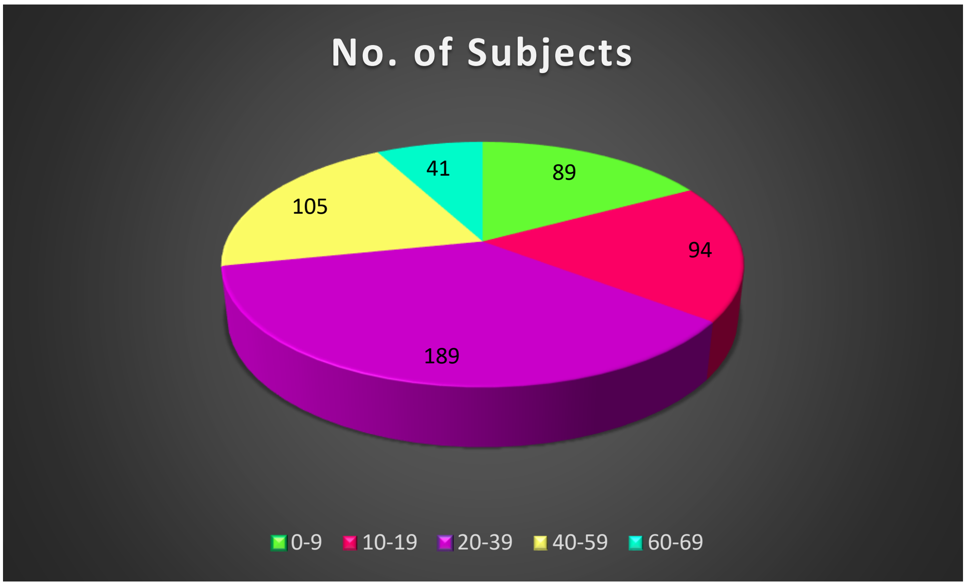


Fig 5.20 Age wise distribution of selected subjects for the study (n=518)

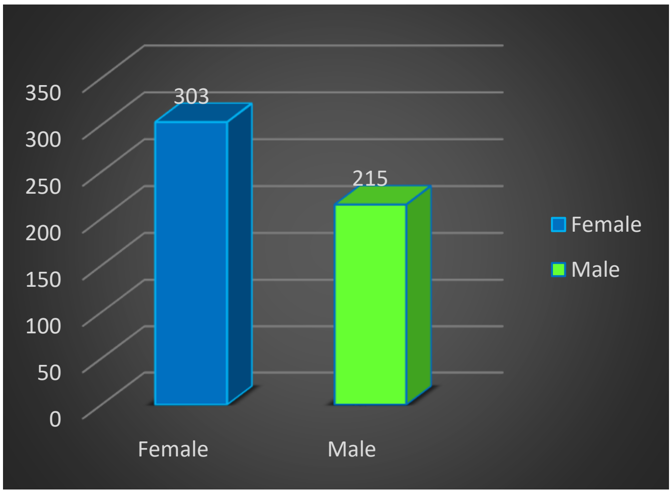


Fig 5.21 Gender wise distribution of selected subjects for the study (n=518)

Data from the consumer acceptability showed a significant difference ($p<0.000$) amongst the likings amongst each age group. Out of all the age groups defined for the study, the packaged *ambil* was liked most by the elderlies above 60 years of age with an average liking of 8.52 out of 9 on the hedonic rating scale. The average liking by other age groups 40-59 years, 20-39 years, 10-19 years and less than 10 years was 7.96, 7.74, 7.71 and 7.56 by respectively (Fig 5.22).

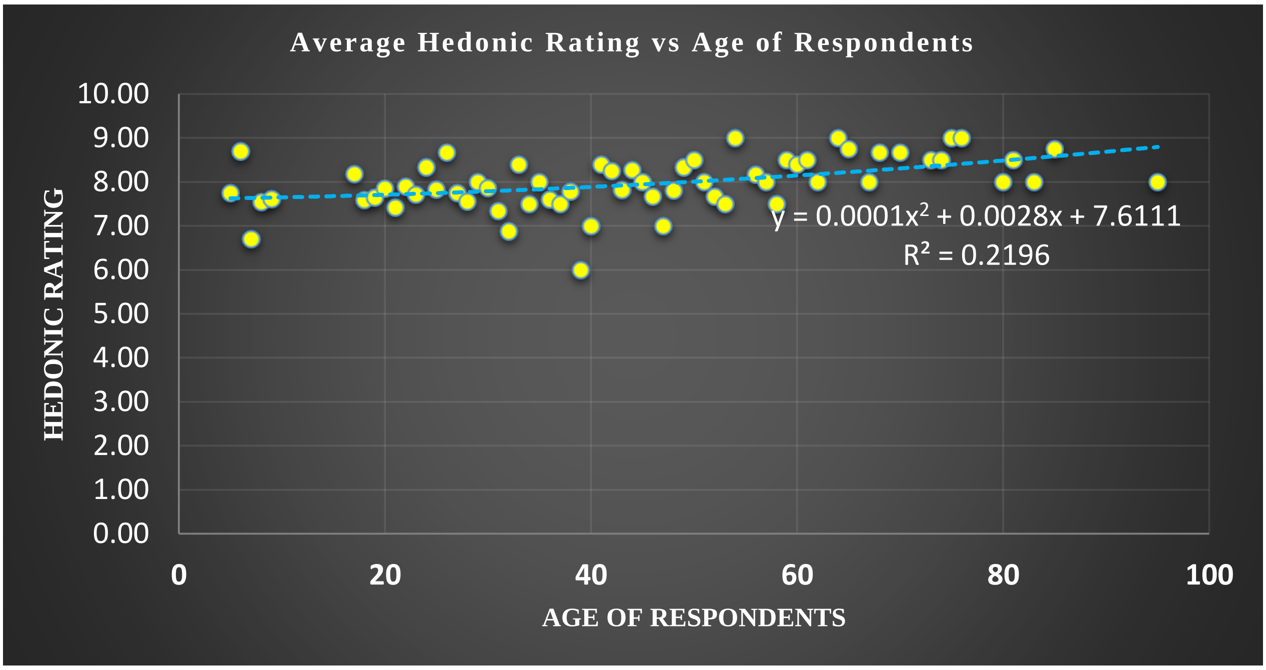


Fig 5.22 Average rate of liking of packaged *ambil* by different age groups

In the age group of less than 10 years 84% of the consumers graded the product 7 and above on hedonic scale, in the age group of 10-19 years 88% of the consumers graded the product 7 and above on hedonic scale, in the age group of 20-39 years 92% of the consumers graded the product 7 and above, for the age group 40- 59 years 95% of the consumers graded the product 7 and above and lastly for the age group of >60 years 98% of the consumers graded the product 7 and above (Fig 5.22 and 5.23).

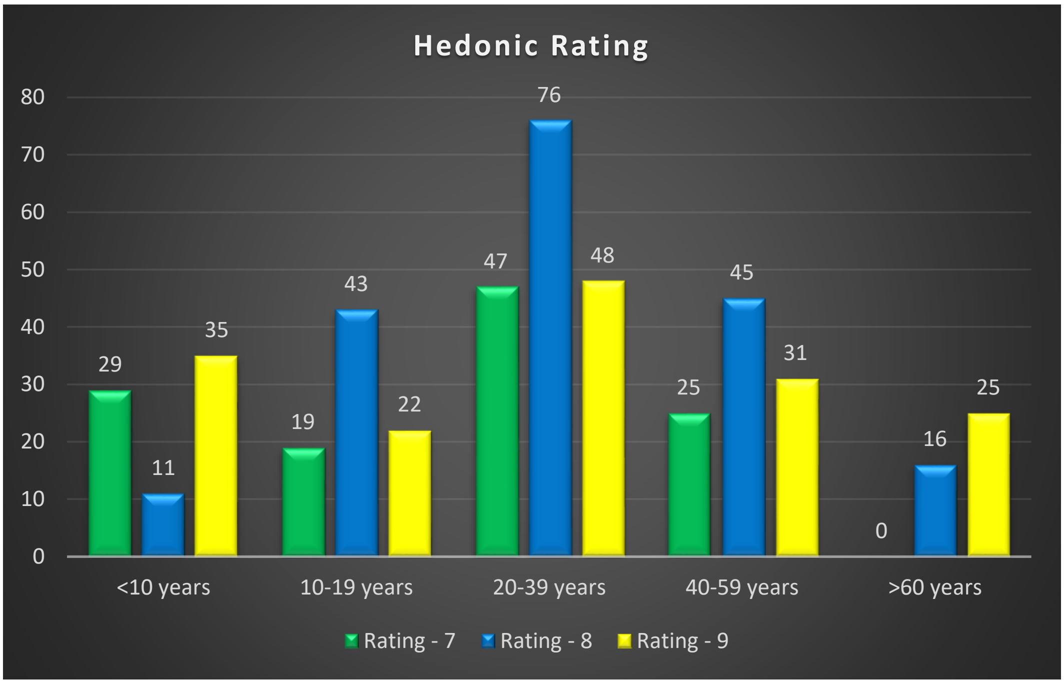


Fig 5.23 Hedonic rating of the product given by different age groups

5.3.5 Market potential packaged rice ambil

Four hundred and twenty nine consumers in the age group of 10-19 years, 20-39 years, 40-59 years and greater than 60 years were selected. The market potential was studied in terms of idea and need of the product, expected cost, desirable flavours and package size of packaged rice ambil.

5.3.5.1 Consumer views on the idea of the packaged rice ambil

Consumer views were studied about the idea of the new health beverage ambil. It was found that more than 50% subjects from all the age groups considered ambil as a good idea and considering ambil as a new product, it sounded interesting. The age group of greater than 60 years were found to be most acceptable to the idea (Table 5.40 and Fig 5.24).

Table 5.40 ***Consumer views on the idea of the packaged rice ambil***

Age Group	Great Idea	Sounds Interesting	Neutral	Chi Square (X2)	p-value
10-19 (n=95)	40 (42)	48 (51)	7 (7)	8.90	0.000
20-39 (n= 186)	92 (49)	78 (42)	16 (9)		
40-59 (n=106)	52 (49)	39 (37)	15 (14)		
>60 (n=42)	20 (48)	21 (50)	1 (2)		

Note: Figures in parenthesis indicate percentages

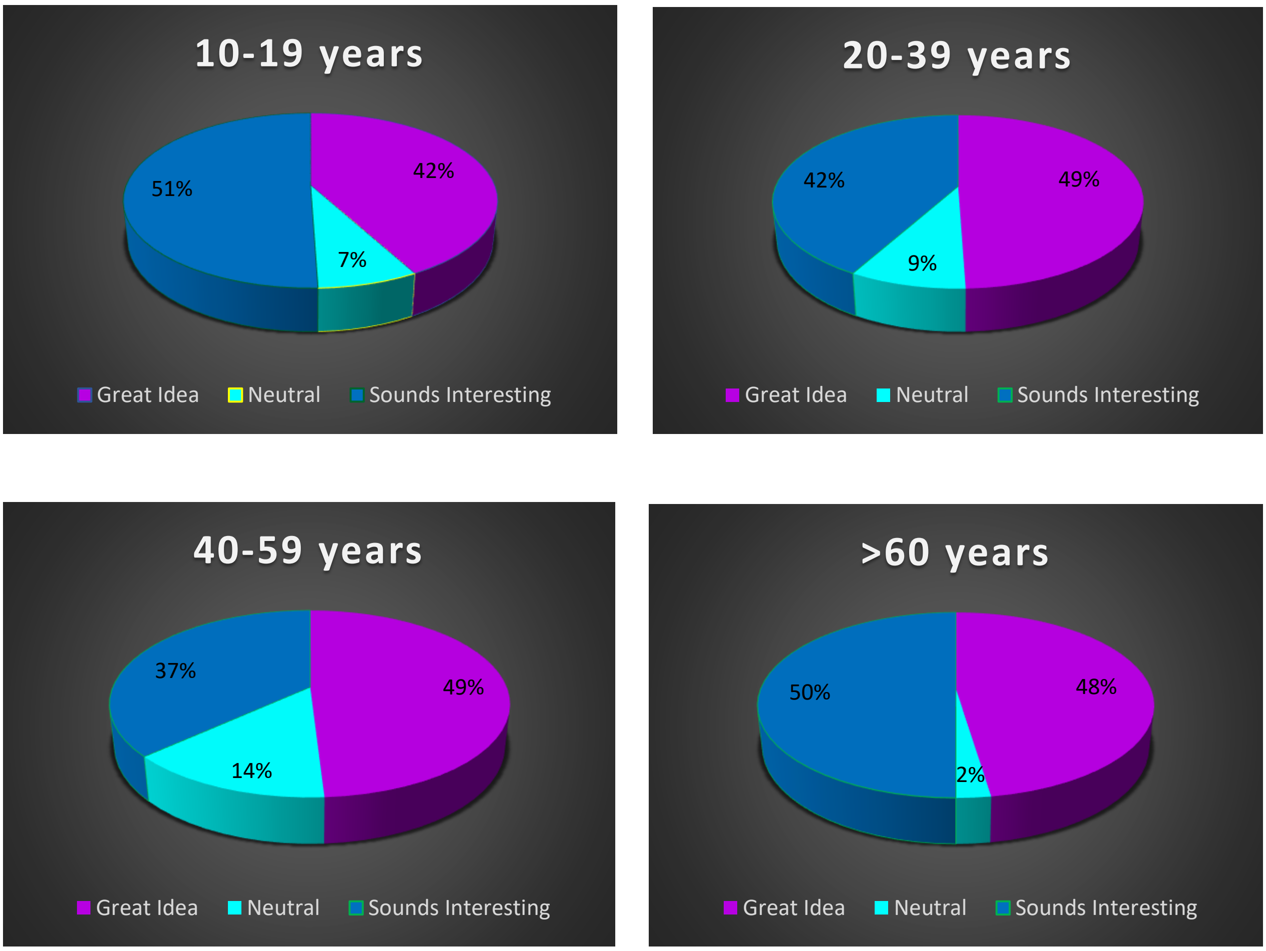


Fig 5.24 Consumer liking of the idea of *ambil*

5.3.5.2 Consumer views on need of the packaged rice ambil commercially

Commercial need of the product was studied and it was seen that the responses varied amongst the age groups (Table 5.41 and Fig 5.25). The commercial need of the product as expressed amongst the age groups depicted that the elderlies (62%) and teenagers (58%) were more open and willing as compared to other age groups. The response from the teenagers may be attributed due to their willingness to experiment with new flavours and drinks.

Table 5.41 ***Consumer views on need of the packaged rice ambil commercially***

Age Group	Yes	No	May Be	Chi Square (X2)	p-value
10-19 (n=95)	55 (58)	9 (9)	31 (33)	10.89	0.000
20-39 (n= 186)	95 (51)	36 (30)	55 (30)		
40-59 (n=106)	53 (50)	25 (24)	28 (26)		
>60 (n=42)	26 (62)	3 (7)	13 (31)		

Note: Figures in parenthesis indicate percentages

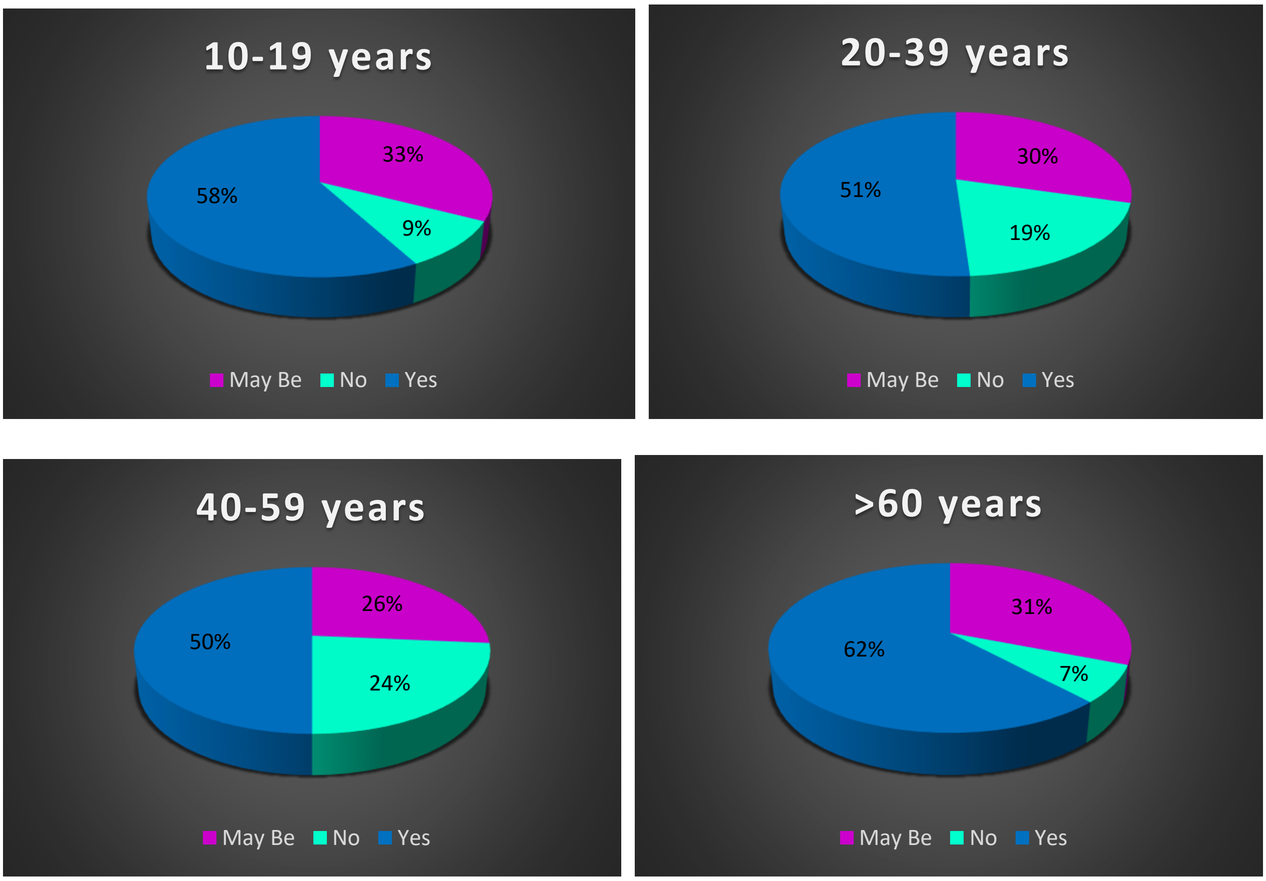


Fig 5.25 Consumer views on need of the packaged rice ambil commercially

5.3.5.3 Consumer preference for purchase of the packaged rice ambil

Consumer preference for purchase of the product was studied if commercially available. More than 90% of the subjects were in favour of buying the product if available commercially (Table 5.42 and Fig 5.26).

Table 5.42 Consumer preference for purchase of the packaged rice ambil

Age Group	Yes	No	May Be	Chi Square (X2)	p value
10-19 (n=95)	88 (93)	6 (6)	1 (1)	3.39	0.000
20-39 (n= 186)	173 (93)	9 (5)	4 (2)		
40-59 (n=106)	100 (94)	4 (4)	2 (2)		
>60 (n=42)	38 (90)	4 (10)	0 (0)		

Note: Figures in parenthesis indicate percentages

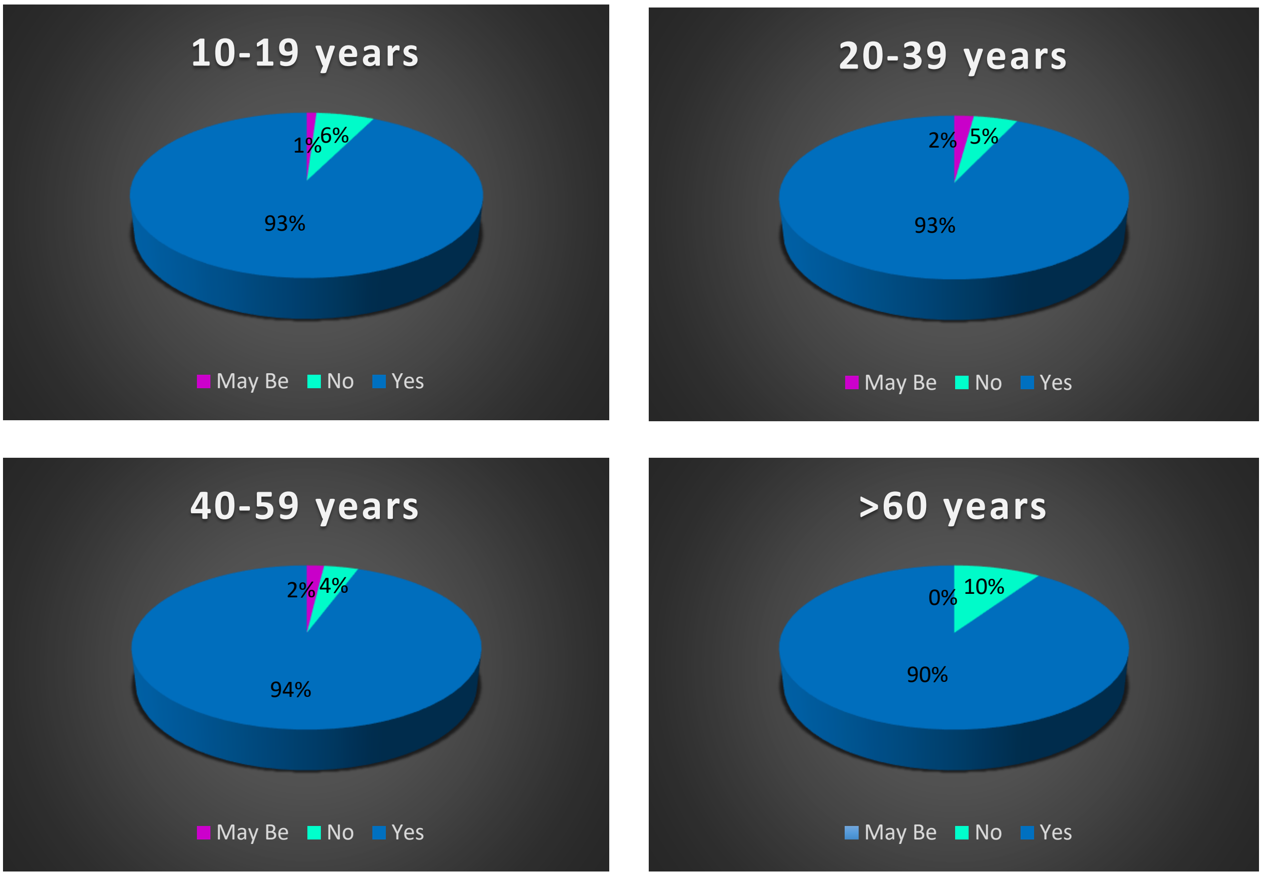


Fig 5.26 Consumer preference for purchasing the packaged rice ambil

5.3.5.4 Consumer preference for cost of the packaged rice ambil

Results depicted in Table 5.43 and Fig 5.27 reveals that the consumers were willing to pay for the packaged ambil if commercially available. Preferred price range was found to be between Rs. 10-15 as suggested by 40-45% of the customers. However, some customers were even willing to pay in the price range of Rs. 15-20 (39%) and even more.

Table 5.43 ***Consumer preference for cost of the packaged rice ambil***

Age Group	<10 Rs.	Rs 10 - 15	Rs 15 - 20	Rs 20 - 25	Rs 25 - 30	Chi Square (X2)	P value
10-19 (n=95)	15 (16)	42 (45)	26 (28)	6 (6)	5 (5)	19.37	0.000
20-39 (n= 186)	17 (9)	84 (45)	63 (34)	14 (8)	8 (4)		
40-59 (n=106)	10 (9)	40 (38)	41 (39)	10 (9)	5 (5)		
>60 (n=42)	12 (29)	17 (40)	12 (29)	1 (2)	0 (0)		

Note: Figures in parenthesis indicate percentages

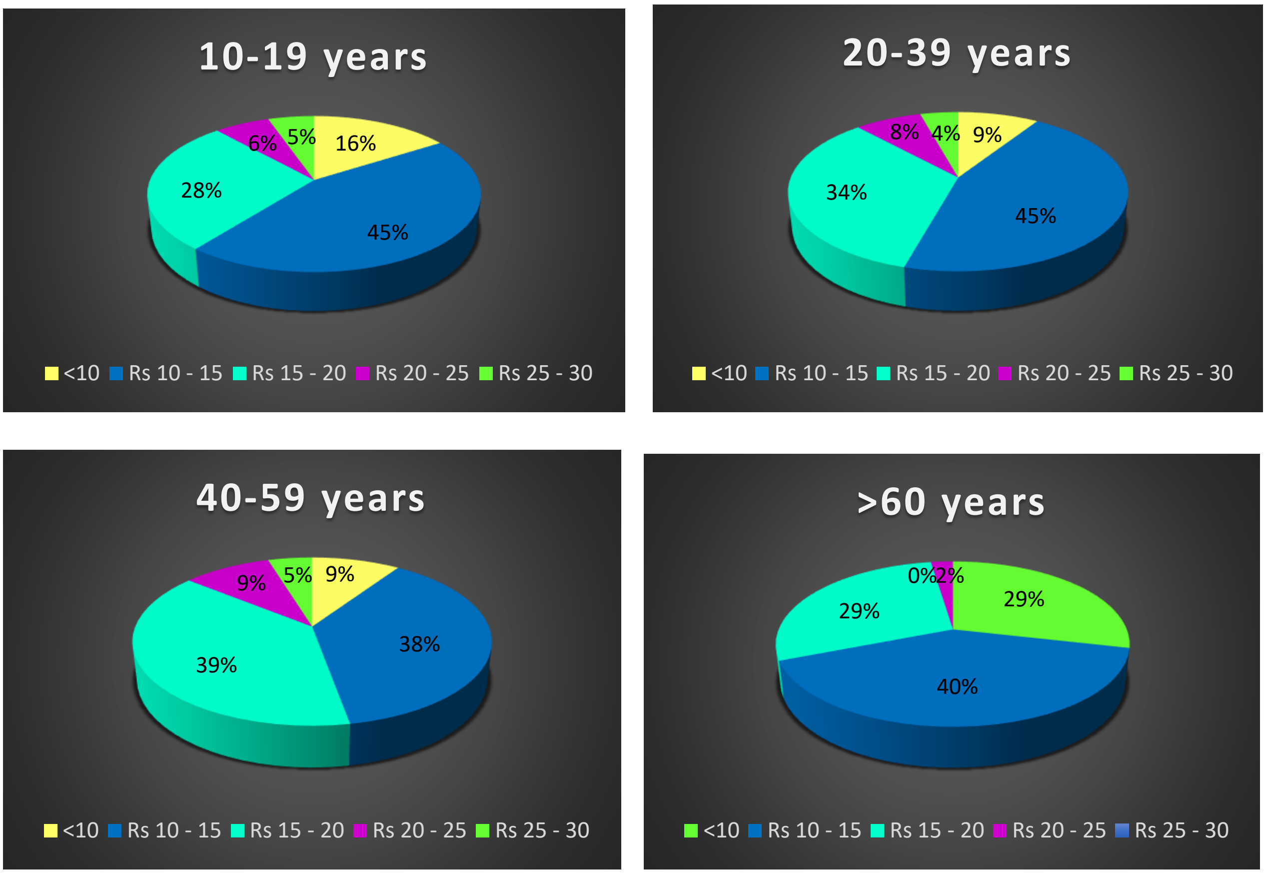


Fig 5.27 Consumer preference for the cost of packaged rice ambil

5.3.5.5 Consumer preference to recommend the packaged rice ambil to others

Results depicted in Table 5.44 and Fig 5.28 reveals that 90-94% of the consumers preferred to recommend the product to others, thus depicting their interest and understanding about the need of the product.

Table 5.44 ***Consumer preference to recommend the packaged rice ambil to others***

Age Group	Yes	No	May Be	Chi Square (X2)	p value
10-19 (n=95)	88 (93)	6 (6)	1 (1)	3.39	0.000
20-39 (n= 186)	173 (93)	9 (5)	4 (2)		
40-59 (n=106)	100 (94)	4 (4)	2 (2)		
>60 (n=42)	38 (90)	4 (10)	0 (0)		

Note: Figures in parenthesis indicate percentages

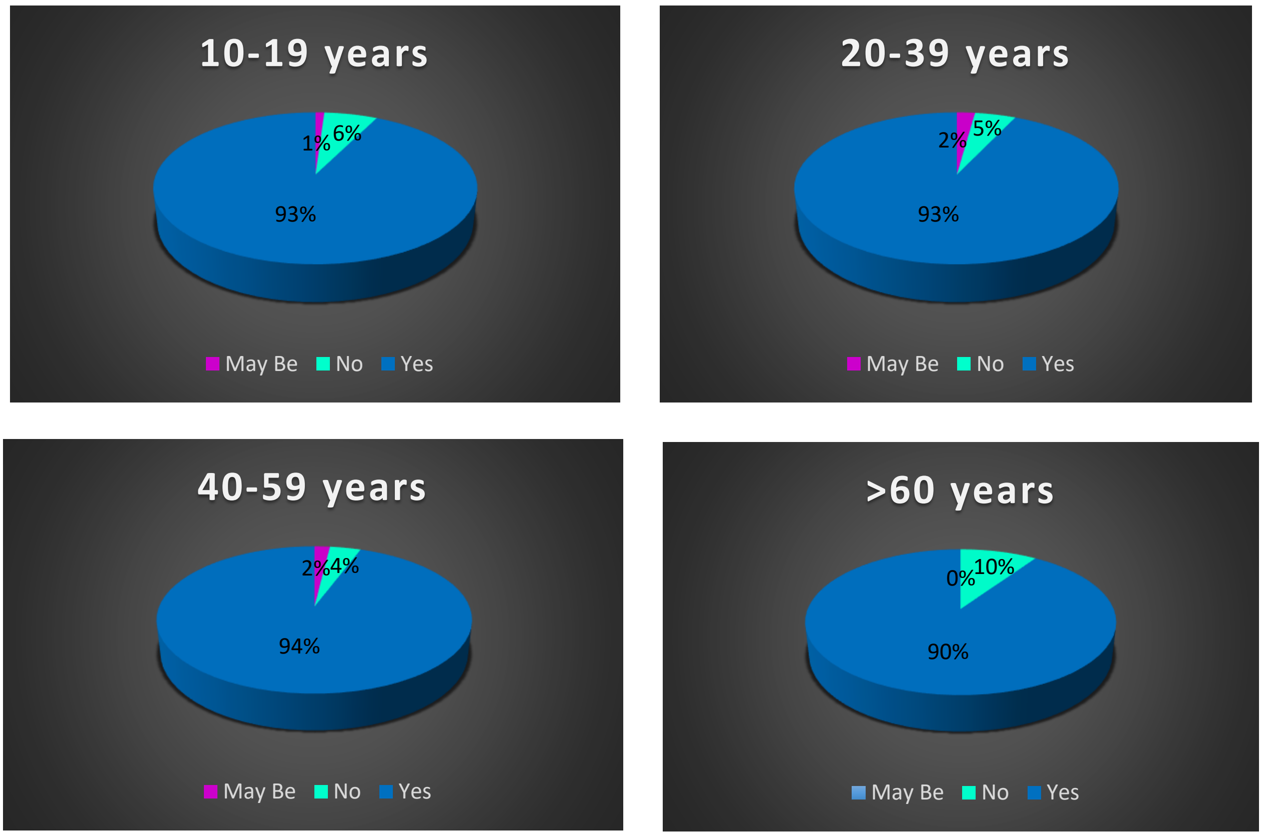


Fig 5.28 Consumer preference to recommend the product to others

5.3.5.6 Consumer preference for flavours of the packaged rice ambil

Consumer preference was determined regarding their preferred flavours of ambil. Choices of flavours were from the commonly available flavours available in the market. The choices varied according to age groups (Table 5.45 and Fig 5.29). Mango and chocolate were the common preferred flavours amongst all the age group except elderlies, who preferred the salt –cumin flavours over all others.

Table 5.45 ***Consumer preference for flavours of the packaged rice ambil***

Age Group	Cardamom	Chocolate	Mango	Pineapple	Rose	Saffron	Salt cumin	Chi Square (X2)	p value
10-19 (n=95)	2 (2)	36 (38)	23 (24)	4 (4)	15 (16)	2 (2)	13 (14)	74.77	0.000
20-39 (n= 186)	6 (3)	55 (30)	36 (19)	16 (9)	26 (14)	17 (9)	30 (16)		
40-59 (n=106)	5 (5)	20 (19)	15 (14)	8 (7)	24 (23)	13 (12)	21 (20)		
>60 (n=42)	1 (3)	1 (3)	0 (0)	2 (4)	6 (14)	10 (24)	22 (52)		

Note: Figures in parenthesis indicate percentages

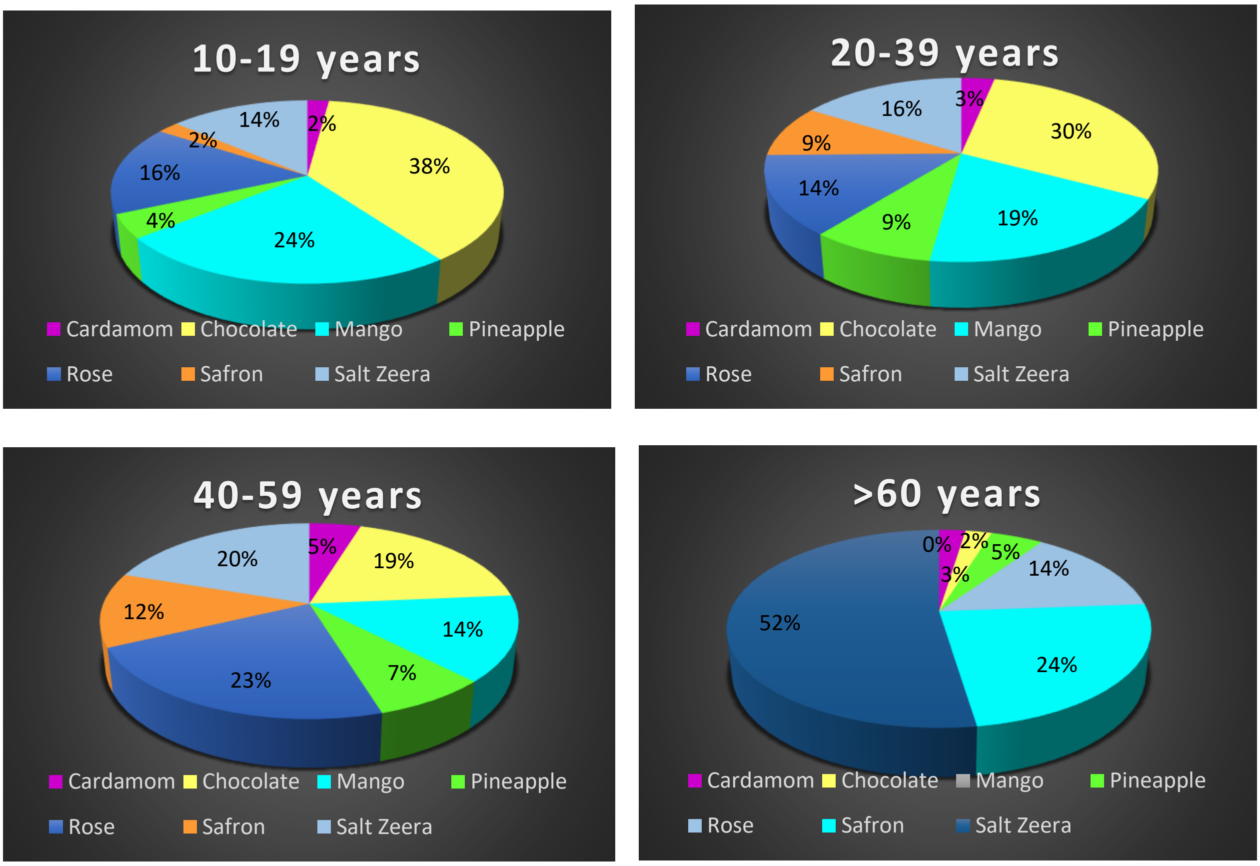


Fig 5.29 Consumer preference for flavours of packaged rice ambil

5.3.5.7 Consumer preference for package size of the packaged rice ambil

Consumer preference for package size of the product was studied, given options were 100ml, 150ml, 200ml, 250ml or >250 ml package size. Most preferred package size amongst all the age groups was 200ml. Not many consumers were in favour of a package size larger than 250ml (Table 5.46 and Fig 5.30).

Table 5.46 ***Consumer preference for package size of the packaged rice ambil***

Age Group	100 ml	150 ml	200 ml	250 ml	> 250 ml	Chi Square (X2)	p value
10-19 (n=95)	24 (25)	26 (27)	30 (32)	11 (12)	4 (4)	20.59	0.000
20-39 (n= 186)	56 (30)	39 (21)	79 (43)	10 (5)	2 (1)		
40-59 (n=106)	29 (27)	23 (22)	48 (45)	6 (6)	0 (0)		
>60 (n=42)	10 (24)	7 (17)	24 (57)	1 (2)	0 (0)		

Note: Figures in parenthesis indicate percentages

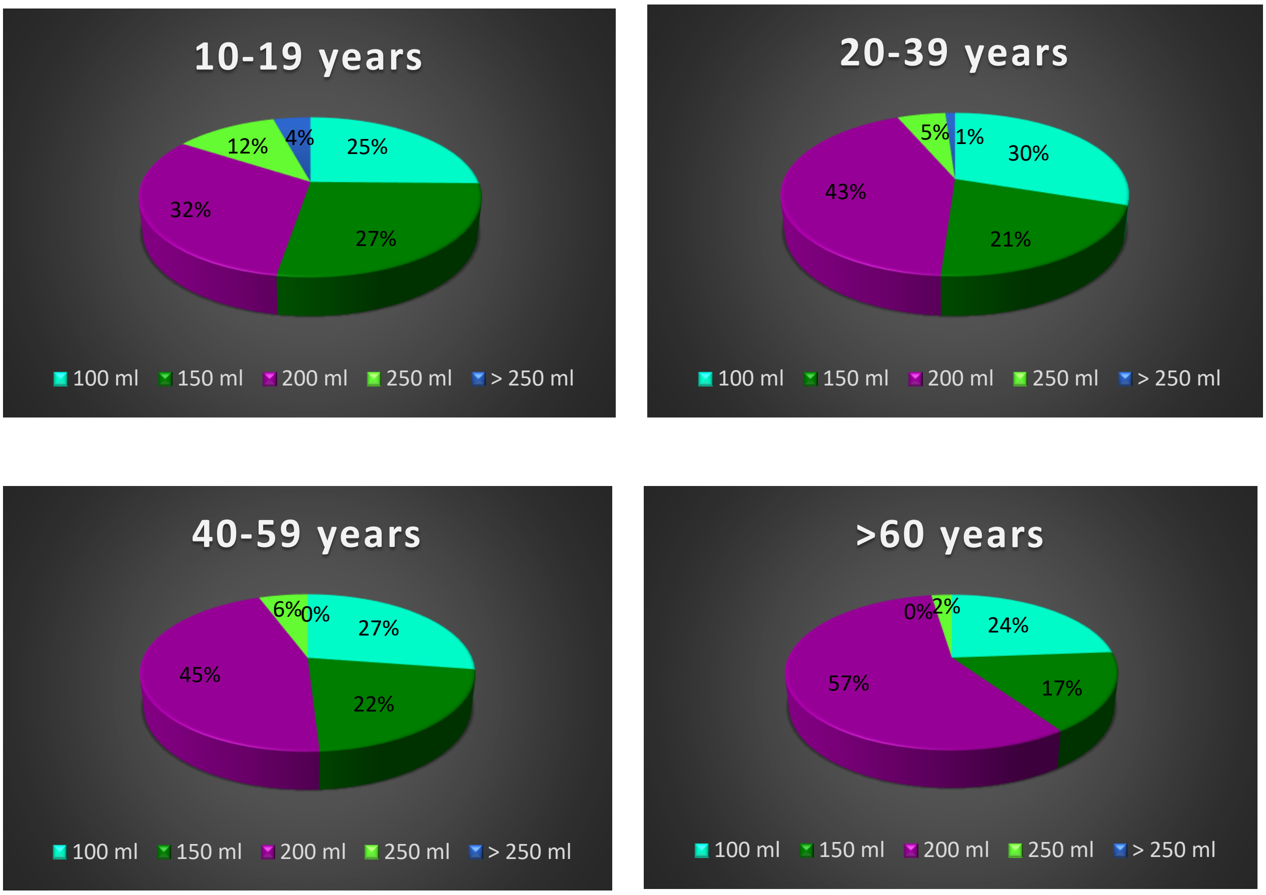


Fig 5.30 Consumer preference for package size of the packaged rice ambil

5.4.2.4. Factors affecting the purchase of health drinks

Consumer's buying behaviour is influenced by cultural, social, personal, psychological factors. In the present study factors affecting the purchase of health drinks by consumers were evaluated. Results depicted (Fig 5.32, Tables 5.47 – 5.55) that one of the major factors affects the purchase are taste (67%) and nutrition (65%). Other factors affecting the purchase behaviour are price (45%), brand (37%) and appearance (29%).

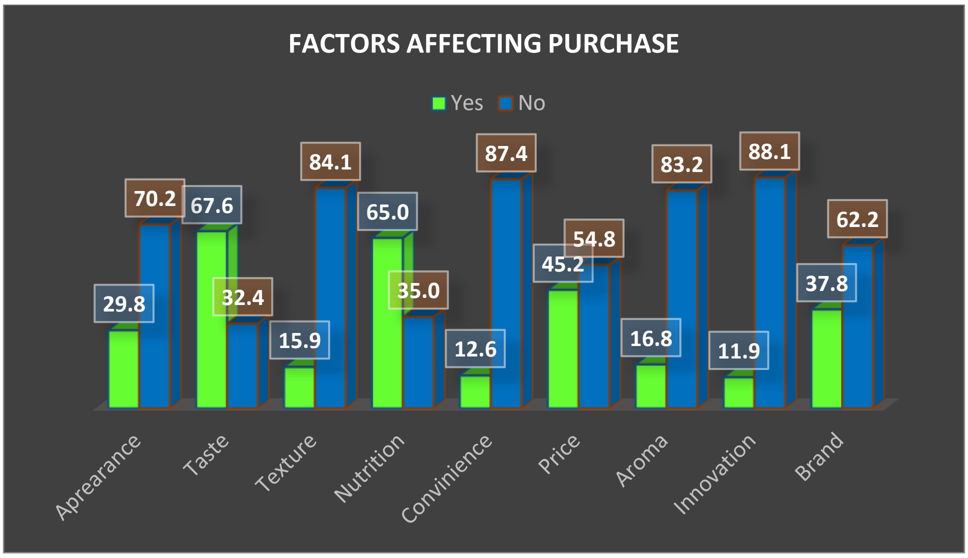


Fig 5.31 Factors affecting purchase of health drinks by the consumers

Table 5.47 Appearance as a factor for purchase of health drinks by the consumers

Age Group	Yes	No	Chi Square (X2)	P value
10-19 (n=95)	20 (19)	75 (81)	21.96	0.000
20-39 (n= 186)	67 (36)	119 (64)		
40-59 (n=106)	39 (36.7)	67 (63.3)		
>60 (n=42)	2 (4.7)	40 (95.3)		

Note: Figures in parenthesis indicate percentages

Table 5.48 Taste as a factor for purchase of health drinks by the consumers

Age Group	Yes	No	Chi Square (X2)	P value
10-19 (n=95)	58 (61)	37 (39)	3.31	0.000
20-39 (n= 186)	133 (71.5)	53 (28.5)		
40-59 (n=106)	70 (66)	36 (34)		
>60 (n=42)	29 (69)	13 (31)		

Note: Figures in parenthesis indicate percentages

Table 5.49 Texture as a factor for purchase of health drinks by the consumers

Age Group	Yes	No	Chi Square (X2)	P value
10-19 (n=95)	12 (12.6)	83 (87.4)	3.56	0.000
20-39 (n= 186)	34 (18.2)	152 (81.8)		
40-59 (n=106)	13 (12.2)	93 (87.8)		
>60 (n=42)	9 (21.4)	33 (78.6)		

Note: Figures in parenthesis indicate percentages

Table 5.50 Nutrition as a factor for purchase of health drinks by the consumers

Age Group	Yes	No	Chi Square (X2)	P value
10-19 (n=95)	63 (66.3)	32 (33.7)	53.75	0.000
20-39 (n= 186)	133 (71.5)	53 (28.5)		
40-59 (n=106)	77 (72.6)	29 (27.4)		
>60 (n=42)	6 (14.2)	36 (85.8)		

Note: Figures in parenthesis indicate percentages

Table 5.51 Convenience as a factor for purchase of health drinks by the consumers

Age Group	Yes	No	Chi Square (X2)	P value
10-19 (n=95)	11 (11.5)	84 (88.5)	6.19	0.000
20-39 (n= 186)	31 (16.6)	155 (83.4)		
40-59 (n=106)	10 (9.4)	96 (90.6)		
>60 (n=42)	2 (4.7)	40 (95.3)		

Note: Figures in parenthesis indicate percentages

Table 5.52 Price as a factor for purchase of health drinks by the consumers

Age Group	Yes	No	Chi Square (X2)	P value
10-19 (n=95)	52 (54.7)	43 (45.3)	9.08	0.000
20-39 (n= 186)	88 (47.3)	98 (52.7)		
40-59 (n=106)	41 (38.6)	65 (61.4)		
>60 (n=42)	13 (30.9)	29 (69.1)		

Note: Figures in parenthesis indicate percentages

Table 5.53 Aroma as a factor for purchase of health drinks by the consumers

Age Group	Yes	No	Chi Square (X2)	P value
10-19 (n=95)	9 (9.4)	86 (90.6)	9.08	0.000
20-39 (n= 186)	42 (22.5)	144 (77.5)		
40-59 (n=106)	14 (13.2)	92 (86.8)		
>60 (n=42)	7 (16.6)	35 (83.4)		

Note: Figures in parenthesis indicate percentages

Table 5.54 Innovation as a factor for purchase of health drinks by the consumers

Age Group	Yes	No	Chi Square (X2)	P value
10-19 (n=95)	10 (10.5)	85 (89.5)	9.25	0.000
20-39 (n= 186)	30 (16.1)	156 (83.9)		
40-59 (n=106)	11 (10.3)	95 (89.7)		
>60 (n=42)	0 (0)	42 (100)		

Note: Figures in parenthesis indicate percentages

Table 5.55 Brand as a factor for purchase of health drinks by the consumers

Age Group	Yes	No	Chi Square (X2)	P value
10-19 (n=95)	39 (41)	56 (59)	0.98	0.000
20-39 (n= 186)	66 (35.4)	120 (64.6)		
40-59 (n=106)	40 (37.7)	66 (62.3)		
>60 (n=42)	17 (40.4)	25 (59.6)		

Note: Figures in parenthesis indicate percentages

Thus it can be concluded that maximum consumers consider taste and nutrition as the major factors for purchase of any commercially available health drink. Other factors affecting the purchase behaviour are price, brand and appearance.

Result highlights of Phase IV

- Frequency of consumption of curd/ buttermilk (95.2%) and fruit juices (93.3%) was observed highest among 40-59 years of age.
- Frequency of consumption of probiotic beverages available commercially was observed highest (52.6%) in the age group of 10-19 years.
- 91% consumers gave the rating >7 which indicates positive acceptability of packaged rice ambil across all age groups, and liked most by the age group of elderlies (98%) followed by 40-59 years (95%) of age.
- Consumer acceptability also depicted that likability of the age group increases with the age with significant difference ($p<0.01$)
- Market potential revealed that the health beverage is needed commercially and consumers were willing to purchase (94%) this product if available in market.
- Preferred price range was found to be between Rs. 10-15 as suggested by 40-45% of the customers. However, some customers were even willing to pay in the price range of Rs. 15-20 (39%) and even more as well as were willing to recommend it to other people as well.
- Mango and chocolate were the preferred flavours amongst age group 10-39 yrs. Salt cumin and rose flavour was preferred by all consumers above 40 yrs of age.
- Most preferred package size amongst all the age groups was 200ml.
- Major factors affecting consumer purchase behaviour for health drinks are taste, nutrition, price and brand.

Discussion

Acceptance of a food is basically the result of the interaction between food and man at a certain moment (Shepherd, 2007). Food characteristics (chemical and nutritional composition, physical structure and properties), consumer characteristics (genetic, age group, gender, physiological and psychological state) and those of the consumer's environment (family and cultural habits, religion, education, fashion, price or convenience) the influence of consumers´ decision to accept or reject a food (Shepherd, 2007; Shepherd and Sparks, 2011). Several studies have been conducted to examine how consumers evaluate different product attributes in numerous food products. Health, nutrition, taste, price, convenience are some of the criteria consumers use to determine which product is more attractive (Adinsi *et al*, 2015).

India is one of the largest economies in the world in terms of purchasing power. With total household consumption expected to increase four-folds from 2005 to 2025, India is poised to emerge as the world's largest consumer market by 2030, with an aggregate spending of USD 13 trillion. More than 50% of India's current population is below the age of 25 and over 65% is below the age of 35. The rise in health-consciousness is also reflected in the growth of health care spending, which is growing at a CAGR of 14 percent, from 5.5 percent of the GDP in 2009 to 8 percent in 2012. The figure is expected to grow to US$ 280 billion by 2020 (Bedi and Paul, 2013).

Many of the health drinks available in the Indian market have their origins in traditional Ayurveda or the indigenous household beverages. The present research study also involves a traditional indigenous household beverage which was modified and packaged at industry to understand the consumer acceptance and willingness to purchase. The health beverage *Ambil* was liked by all the age groups and majority of the subjects felt there is need of such health beverages at commercial level. This may be due to the increasing interests of consumers towards a healthy lifestyle which leads to willingness of accepting new innovative health products or functional foods. Many studies have proven the importance of health influences the choice of these foods and, specifically, how the consumer's healthy lifestyle influences the attitude and willingness of consumption of these foods (Ozen *et al*, 2014; Ozen *et al*, 2012; Aschemann and Hamm, 2010; Boluda and Kapilla, 2017).

The purchasing ability and buying behavior of Indian consumers have increased with the increase in knowledge. While all segments of the beverage market are evolving, the growth seems to be directed more towards healthy, light and low-calorie drinks. The desire for health and concern for ill-health has driven consumers towards these drinks. Healthier product alternatives, including drinks specifically designed to provide energy, nutrition and health to the human body, are among becoming top priority (Sloan, 2003). The consumers for the health drinks are people from the different walks of life but the majority of them come from the younger and older generations. Present study indicated that the subjects were willing to pay for the drink if available commercially in the price range of 10-20 Rs and some even more than that. Similar study was conducted for the acceptability and purchase of health beverages in Bangalore (Tejaswini and Nagaraja, 2013) the study documented that 30 per cent of the consumers (n= 60) agree which may be due to kinds of consumers who try to buy a product even at higher prices. Dave and Palival, 2016 had also documented similar results on consumer perception of beneficial malted health food drinks in Udaipur city.

The buying behavior of fruit juice consumers was investigated. It was recognized that the consumer buying behavior was mainly influenced by the quality of the product, in which the most important attribute was product's taste (9.6), followed by nutritional value (8.9), odor (8.2) and price (8.0) on a scale of 1 to 9. **(**Krasaekoopt and Kitsawad, 2010).

With the advent of urbanisation and globalisation, rise of double income families, and tremendous stress in today's world, women have less time to spend in kitchen. Thus, family is deprived of fresh and nutritious diet. In order to combat nutritional deficiencies, more and more nuclear families are driven towards convenient and nutritious foods. This has led to the emergence of functional food sector in India. Moreover, as people are living longer (ageing population), with lengthened years, comes increased threat of chronic diseases and from a socio economical viewpoint, countries are facing financial difficulties in escalating medical care costs due to the increase in chronic diseases and the expansion of life spans. Thus, it provides a cost effective alternative rather than to invest in medicine. Moreover, a vast majority of people has a positive image of healthy food and agrees that food and nutrition have a positive impact on long-term and current health (Landstrom *et al*. 2007). The observed growth in this market is driven largely by consumers aim for a healthier lifestyle through a dietary approach.

Frequency of consumption of curd and buttermilk was studied in the present study. Results depicted that majority of people (84-95%) in all the age groups consume curd or buttermilk on daily basis. These results are similar to the results documented in the study conducted by Priyanka and Mallika, 2013 where 626 volunteers (81.3%) were consuming curd since childhood. Curd and buttermilk/ lassi consumption pattern was studied in the sample population of North, Central and south India. More than one out of five sample population never consumed curd, while majority consumed 1 portion daily. According to the report of Milk and Dairy Products in India – Production, Consumption and Exports, it was estimated that almost 10 per cent of liquid milk bought by households is converted into curd, as majority of Indian households consume curd on daily basis (Chawla *et al*, 2009). In another study conducted in Maharashtra, analysis showed that 95.83 per cent of households regularly consumed dairy foods like curd, lassi, buttermilk etc. (Govindrao, 2012). Deaton *et al*., (2009) states that buttermilk widely available often free of cost, in many villages of India, particularly in the north-western region. Consumption of 1 portion of curd daily was more common in age 45–54 years, which is similar to the results of our study. A contradictory result was found for buttermilk which stated that one out of four participants never drink buttermilk/lassi while only 14% drink 1 portion daily (Satija *et al*, 2013; Kumari *et al*, 2018).

The consumption pattern of fruit juices in the present study documented 93%, 89% and 84% in the age groups of40-59yrs, 20-39yrs and 10-19yrs respectively. This may be due to rise in the disposable income, people adopting Western culture, health awareness and import of fruits to India are among the top most factors to drive the juice business in India (Granqvist and Ritvala, 2016). The rising number of health-conscious consumers is giving a boost to fruit juices; it has been observed that consumers are shifting from fruit-based drinks to fruit juices as they consider the latter a healthier breakfast/snack option.

Consumption of probiotic beverages was studied in the present study, and it was observed that the consumption rate was lesser as compared to curd, buttermilk or fruit juices. Results documented that the maximum consumption was 52% in the age group of 10-19yrs, followed by 37% and 31% in the age group of 20-39 yrs and 40-59yrs. This may be due to lack of knowledge and awareness amongst the consumers for probiotics. Thirty eight percent consumer had never consumed any kind of probiotic beverages available in market. A study conducted in Punjab documented that out of 220 adult respondents only 60.5% of respondents were familiar with the word probiotic out which only 36.8% have ever consumed probiotic. The consumption rate is higher in the age group of 10-19 yrs which may be due to their education and exposure to new innovations and updated knowledge about health products (Swetaa *et al*, 2018; Khongrangjem *et al*, 2017, Antony and Bhatti, 2015). A study was conducted on the nutrition awareness of the students of Government Degree College, Jammu and Kashmir. Sample was selected randomly of 100 students of various departments and Nutrition awareness of the students was measured regarding nutrition and health status, health foods etc, results documented that 76% of the students have good and excellent knowledge about health foods and other parameters studied (Ahmad *et al*, 2018).

Conclusion

- *Packaged rice ambil had a good acceptability amongst all the age groups and has a good market potential. Data from the consumer acceptability trials also depicted that likability of the ambil increases significantly with age.*
- *Major factors affecting consumer purchase behaviour are taste and nutrition followed by price, brand and appearance.*