CHAPTER-5
RESULTS AND DISCUSSION

The results of the present study entitled **"Extraction of Xylooligosaccharides (XOS) from agricultural waste, determining its prebiotic properties and organoleptic qualities of Indian traditional foods upon its addition"** are presented and discussed in this chapter. These results are presented in to three main phases according to the objectives of the study.

PHASE I: Extraction of xylooligosaccharide from selected agricultural wastes.

PHASE II: Determining the prebiotic properties of XOS in terms of bile resistance, acid tolerance, growth of *Lactobacillus plantarum, Bifidobacterium adolescentis* and *Escherichia coli*; production of short chain fatty acids (SCFA) such as acetate, butyrate and propionate.

PHASE III: Organoleptic evaluation of XOS added *Prawn Patia, Paneer Butter masala, Black Rice Kheer* and *Gajar Ka Halwa* with 5g, 8g and 10g XOS.

PHASE I: Extraction of xylooligosaccharide from selected agricultural wastes.

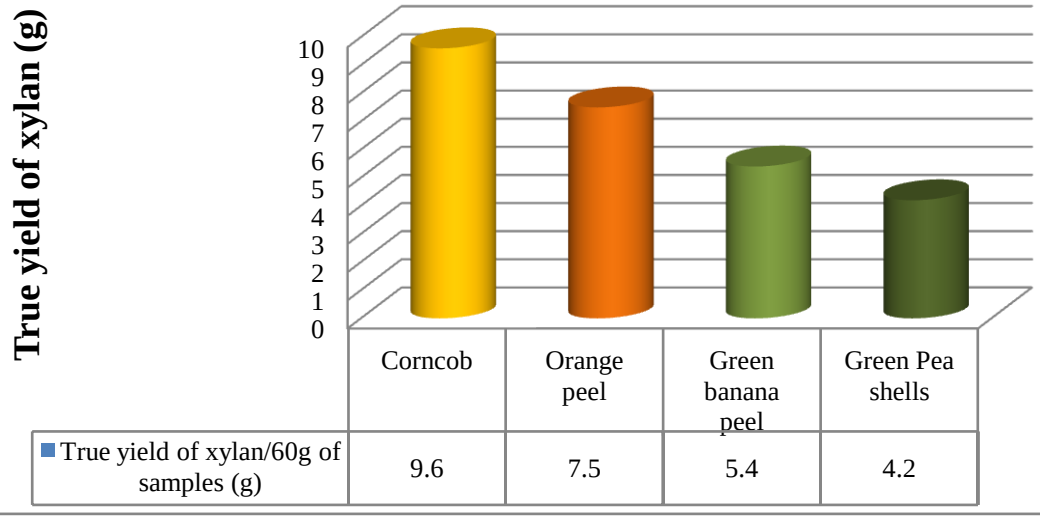
In India, 620million tons of agricultural and agro-industrial residues are being generated approximately (Singh et al, 2017). Around seventy percent of these agricultural wastes are used as fodder, fuel for domestic and industrial sectors etc. (MNRE in association with Indian Institute of Science, Bangalore, 2014). Therefore, an estimated amount of 120-150 million tons of agro wastes or residues remains as a surplus per year and can potentially be used to produce various value added products like bio fuels, animal feeds, chemicals, enzymes etc. (Saha, 2003; Goldman, 2009).

There is a huge scope for the value addition and utilization of these agricultural waste or residues for food applications such as production of XOS, xylitol and xylose (Aachary et al, 2009).Therefore, this phase of the research work was undertaken to determine the extent to which XOS can be extracted from the selected agro waste such as orange peel, green banana peel, corn cob and green pea shells.

5.1. Determination of Xylan in selected agro waste

Different levels of XOS yield were determined from xylan of corn cob, orange peel, green banana peel and green pea shells using 4%sodium hydroxide (NaOH). Crude xylan yield was 9.60 g (16.0%), 7.50 g (12.5%), 5.40 g(9.0%), and 4.20 g (7.0%), respectively (Fig 5.1)

Fig 5.1: True yield of xylan from the selected samples



5.2. Enzymatic hydrolysis of Xylan

Pure XOS obtained from the xylan of 60g corn cob, green banana peel, orange peeland green pea shells were 1.8g (18.75%), 1.01g (18.70%), 1.41g (18.80%)and 0.79g (18.80%) respectively at ($p \leq 0.01$) with an optimal condition of 12h incubation time, pH 5.4 at 40°C (Table 5.2).

Therefore, pure XOS obtained from 100g dry powdered samples of corncob, green banana peel, orange peel and green pea shells were 3g (18.75%), 1.68g (18.66%), 2.35 (18.80%), and 1.31g (18.71%) respectively.

Table 5.2: XOS yield by enzymatic hydrolysis of Xylan at different incubation and pH - 5.5 at 40°C.

Incubation time	Corncob(g)	Banana peel(g)	Orange peel(g)	Green pea shells(g)
4h	1.44	0.81	1.13	0.63
6h	1.58	0.89	1.24	0.69
8h	1.70	0.96	1.33	0.75
12h	1.80	1.01	1.41	0.79
F value	11.78	14.84	13.25	15.77
'p' value	0.013**	0.008**	0.010**	0.007**

Significant at *p≤0.05, **p≤0.01, *** p≤0.001, NS- non significant

5.3. Concentration of XOS among all the agro waste samples

Table 5.3 reveals that concentration of XOS was found to be highest for corncob followed by orange peel, green banana peel and green pea shells among all the four agro waste samples.

Table 5.3: Concentration of XOS among all the four agro wastes

Sl.No.	Sample name	Concentration of XOS (mg/ml)
1.	Standard XOS	100
2.	Corncob's XOS	79.41
3.	Green banana peel's XOS	73.50
4.	Orange peel's XOS	74.73
5.	Green pea shell's XOS	71.94

Fig: 5.2.1 (a) – 5.2.5 (c) shows HPLC chromatograms of XOS standard, XOS derived from corn cob, orange peels, banana peels and green pea shells respectively in triplicates.

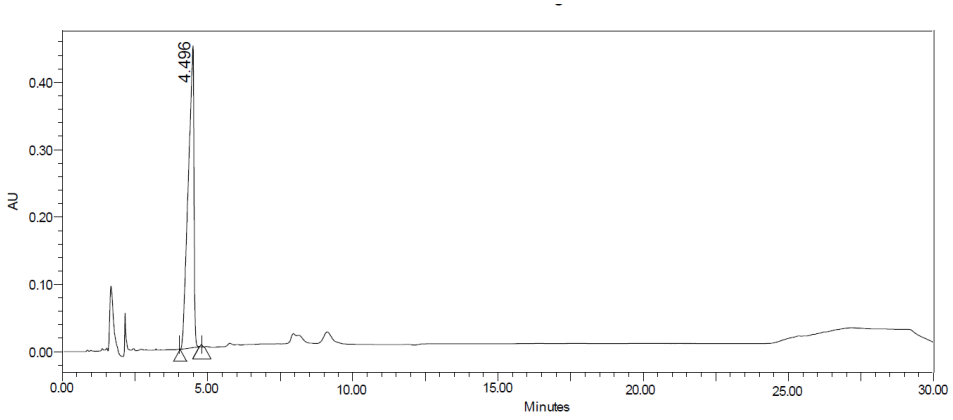


Fig 5.2.1(a): HPLC chromatogram showing peak of XOS standard (1)

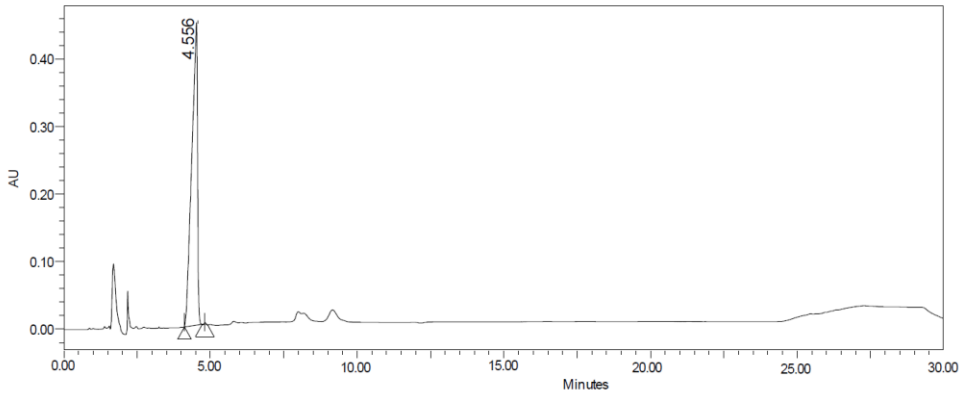


Fig 5.2.1(b): HPLC chromatogram showing peak of XOS standard (2)

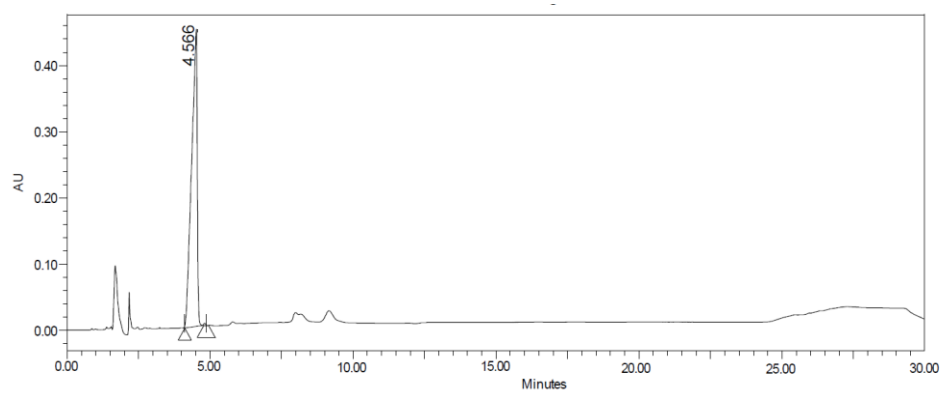


Fig 5.2.1(c): HPLC chromatogram showing peak of XOS standard (3)

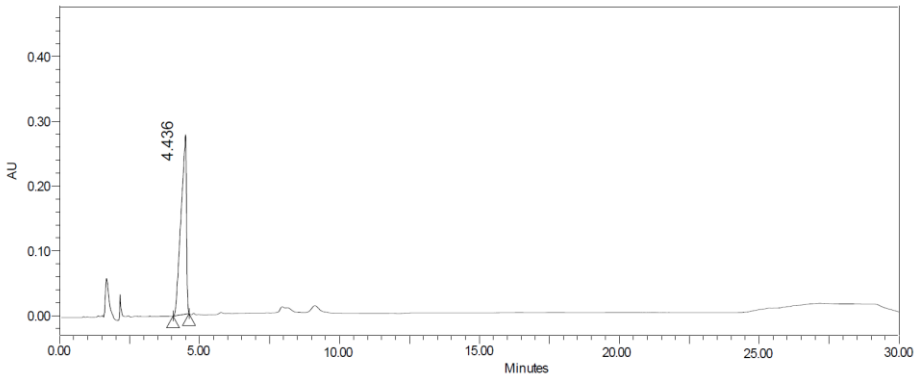


Fig 5.2.2 (a): HPLC chromatogram showing peak of XOS derived from corn cob (1)

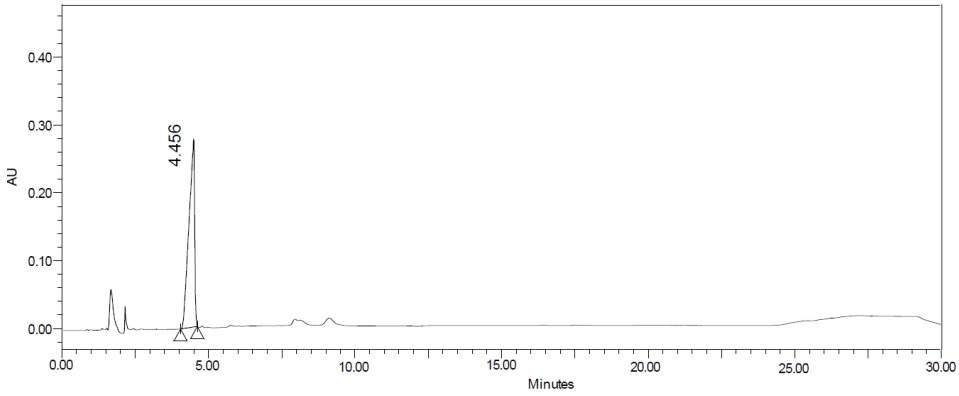


Fig 5.2.2 (b): HPLC chromatogram showing peak of XOS derived from corn cob (2)

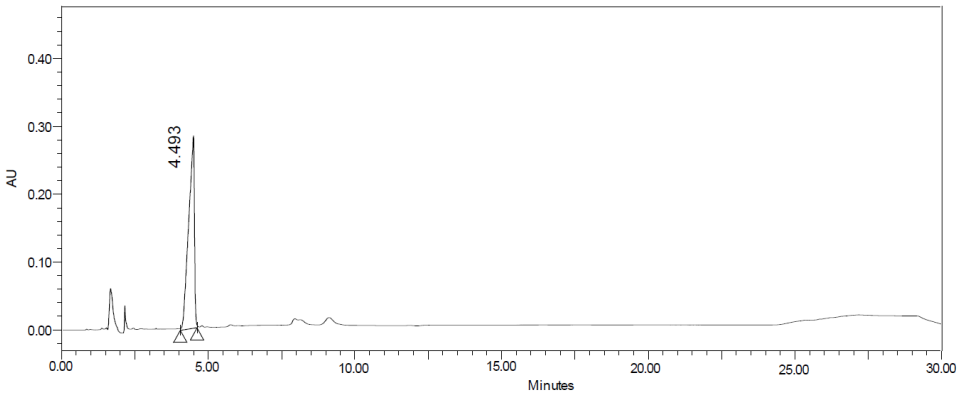


Fig 5.2.2 (c): HPLC chromatogram showing peak of XOS derived from corn cob (3)

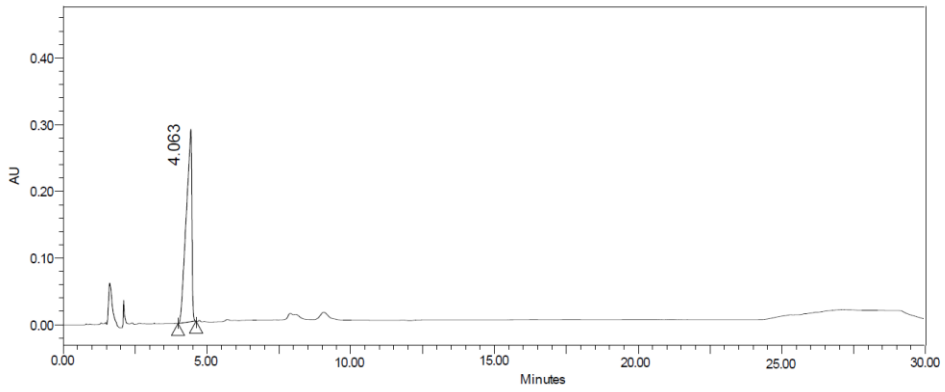


Fig 5.2.3 (a): HPLC chromatogram showing peak of XOS derived from orange peels (1)

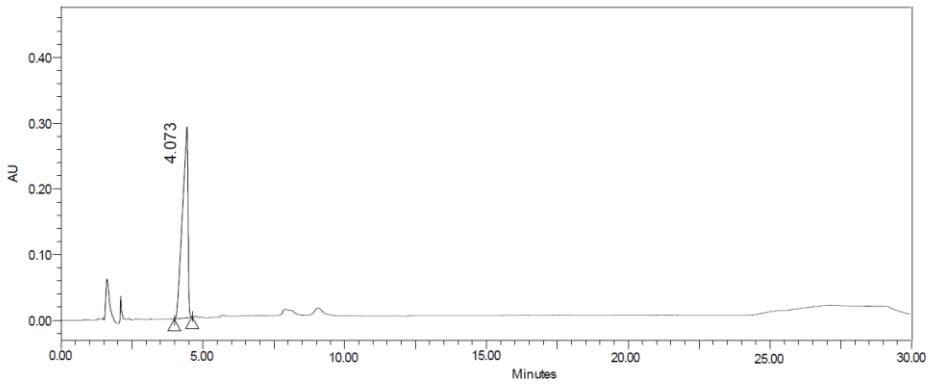


Fig 5.2.3 (b): HPLC chromatogram showing peak of XOS derived from orange peels (2)

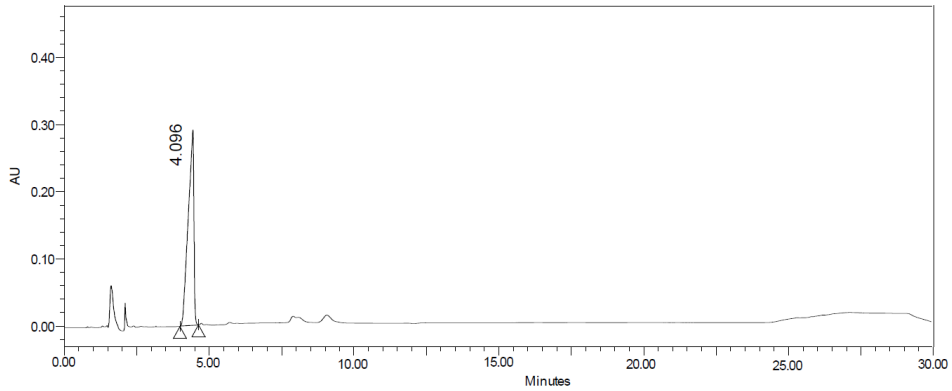


Fig 5.2.3 (c): HPLC chromatogram showing peak of XOS derived from orange peels (3)

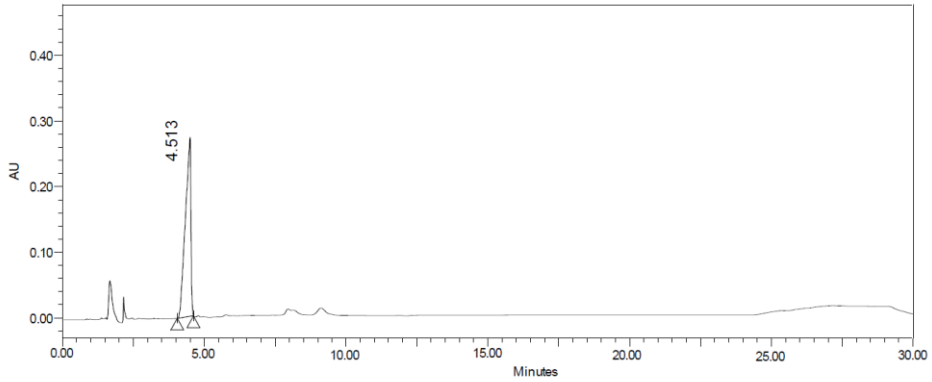


Fig 5.2.4 (a): HPLC chromatogram showing peak of XOS derived from banana peels (1)

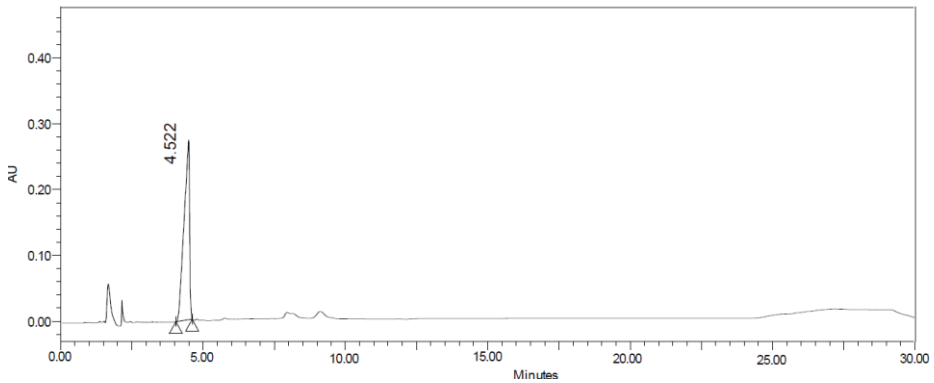


Fig 5.2.4 (b): HPLC chromatogram showing peak of XOS derived from banana peels (2)

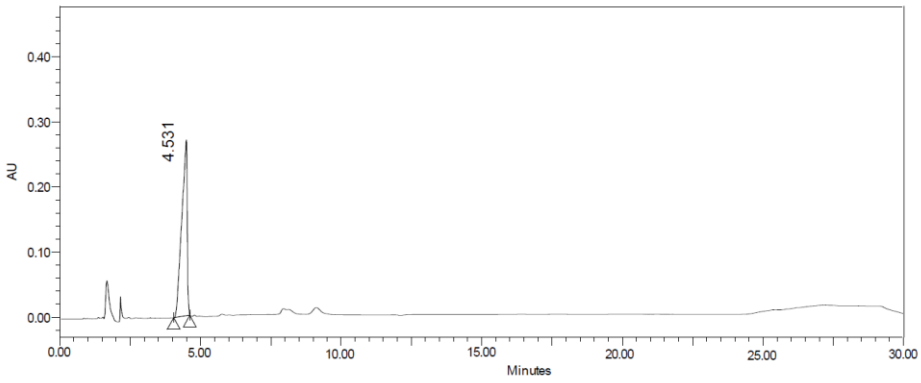


Fig 5.2.4 (c): HPLC chromatogram showing peak of XOS derived from banana peels (3)

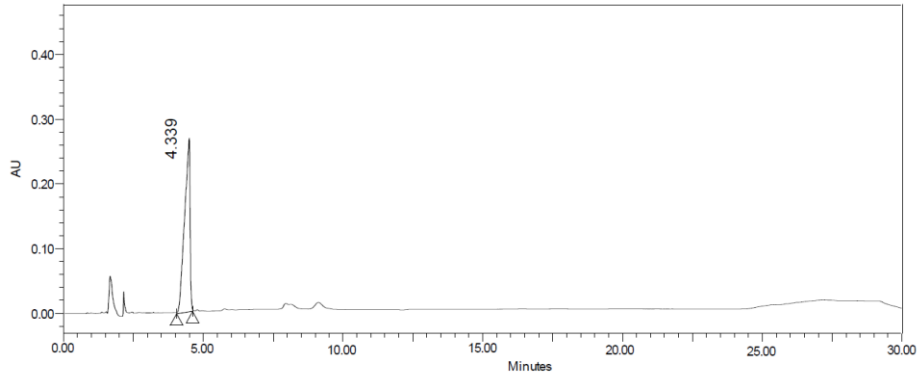


Fig 5.2.5 (a): HPLC chromatogram showing peak of XOS derived from green pea shells (1)

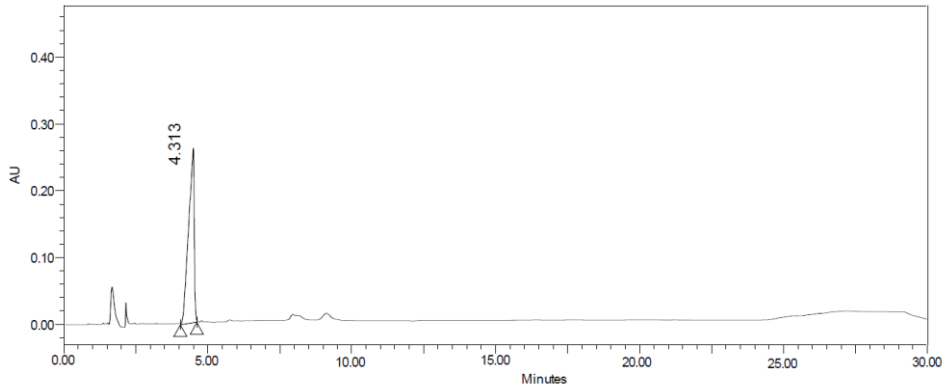


Fig 5.2.5 (b): HPLC chromatogram showing peak of XOS derived from green pea shells (2)

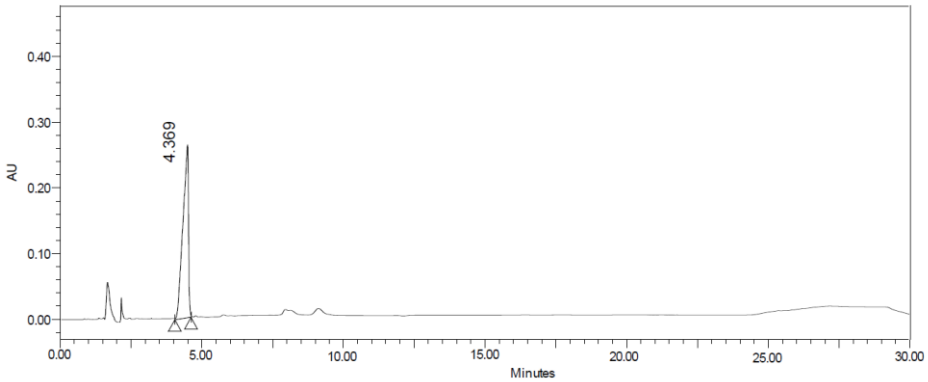


Fig 5.2.5 (c): HPLC chromatogram showing peak of XOS derived from green pea shells (3)

PHASE II: Determining the prebiotic properties of XOS in terms of bile resistance, acid tolerance, growth of *Lactobacillus plantarum, Bifidobacterium adolescentis* and *Escherichia coli*; production of short chain fatty acids (SCFA) such as acetate, butyrate and propionate.

5.4. Bile resistance test of XOS

No degradation of XOS was observed on exposure of XOS to bile at 0h, 1.5h and 3h with bile concentration 0.5%, 1% and 1.5%. The tests were carried out in duplicates.

5.5. Acid tolerance test of XOS

Table 5.4 reveals that XOS recovery was observed to be 100% on its exposure to pH 1.5, 2 and 3 at 0h. At 1.5 h, recovery of XOS was found to be 98.4%, 98.9% and 97.9% at pH 1.5, pH 2 and pH 3 respectively. XOS recovery was 96.2%, 97.3% and 96.3% on its exposure to pH 1.5, pH 2 and pH 3 respectively at 3 h. The tests were carried out in duplicates.

Table 5.4: XOS recovery at different levels of pH

pH	0h	1.5h	3h
1.5pH	100%	98.41%	96.29%
2pH	100%	98.94%	97.32%
3pH	100%	97.93%	96.39%

Fig 5.3.1 (a) – 5.3.9 (b): HPLC chromatograms showing bile resistance of XOS with 0.5%, 1% and 1.5% at 0h, 1.5h and 3h.

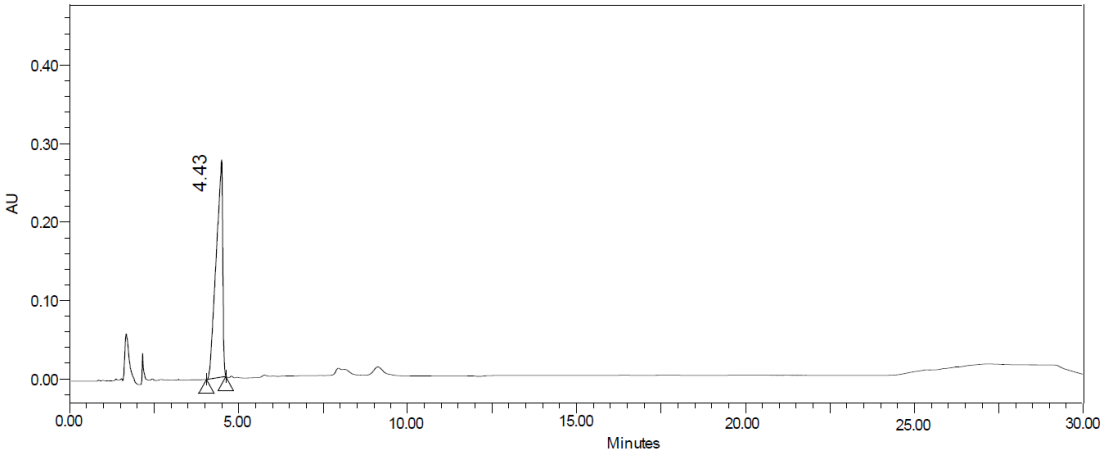


Fig 5.3.1 (a): HPLC chromatograms showing bile resistance of XOS with 0.5% at 0h (1)

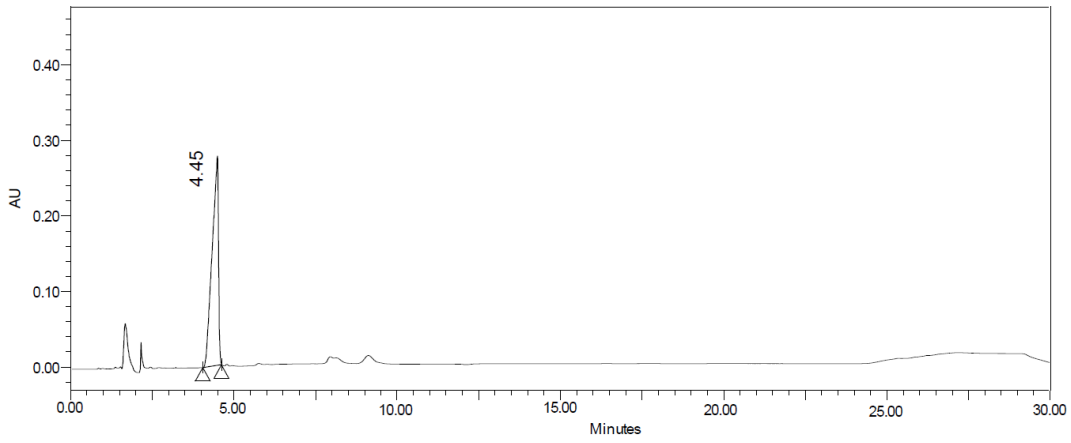


Fig 5.3.1 (b): HPLC chromatograms showing bile resistance of XOS with 0.5% at 0h (2)

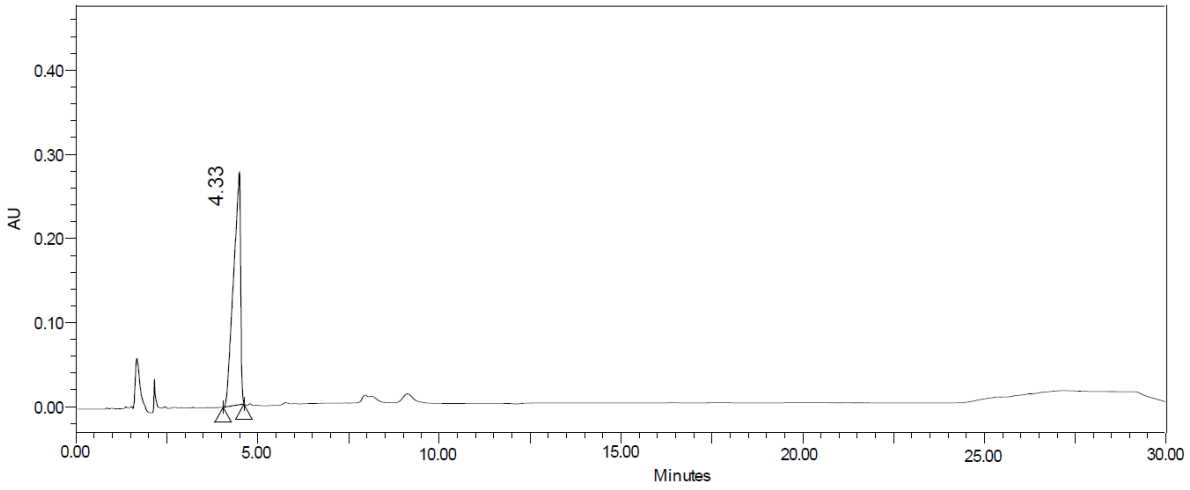


Fig 5.3.2 (a): HPLC chromatograms showing bile resistance of XOS with 0.5% at 1.5h (1)

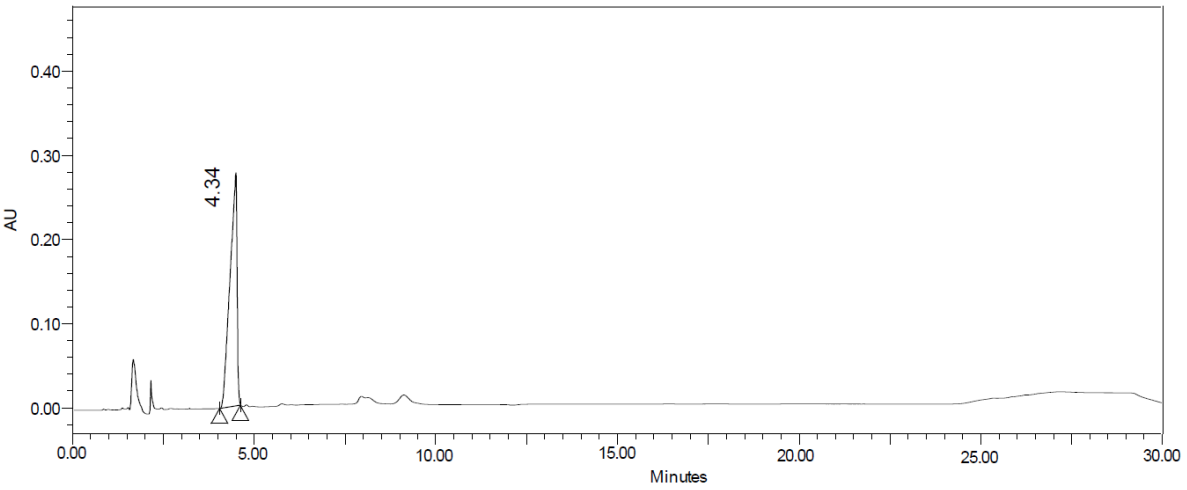


Fig 5.3.2 (b): HPLC chromatograms showing bile resistance of XOS with 0.5% at 1.5h (2)

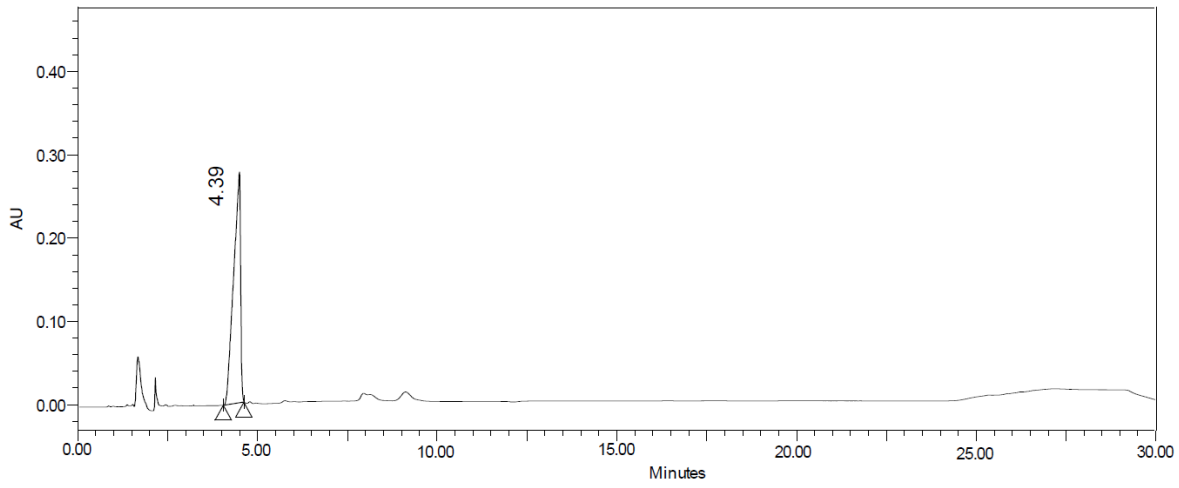


Fig 5.3.3 (a): HPLC chromatograms showing bile resistance of XOS with 0.5% at 3h (1)

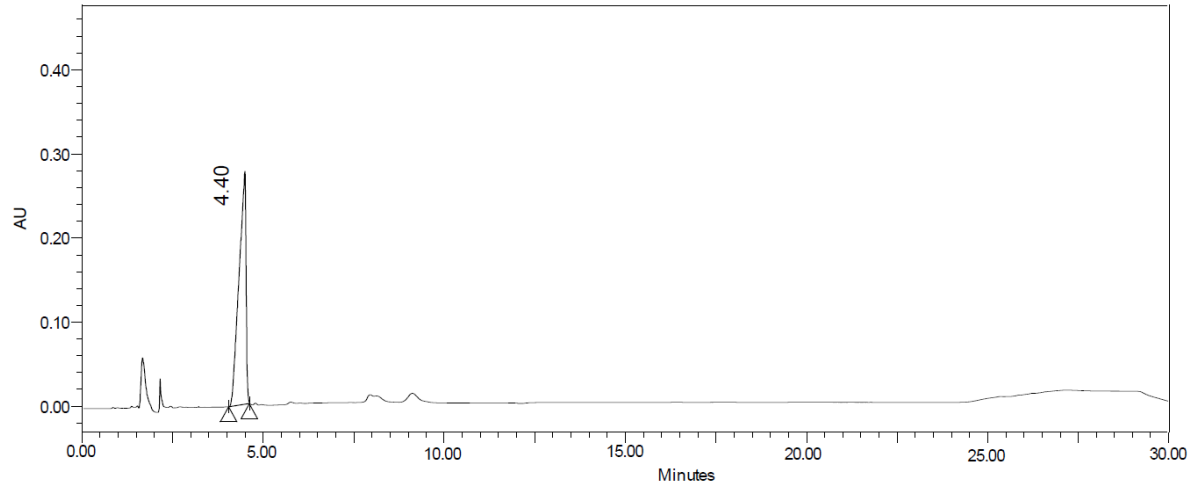


Fig 5.3.3 (b): HPLC chromatograms showing bile resistance of XOS with 0.5% at 3h (2)

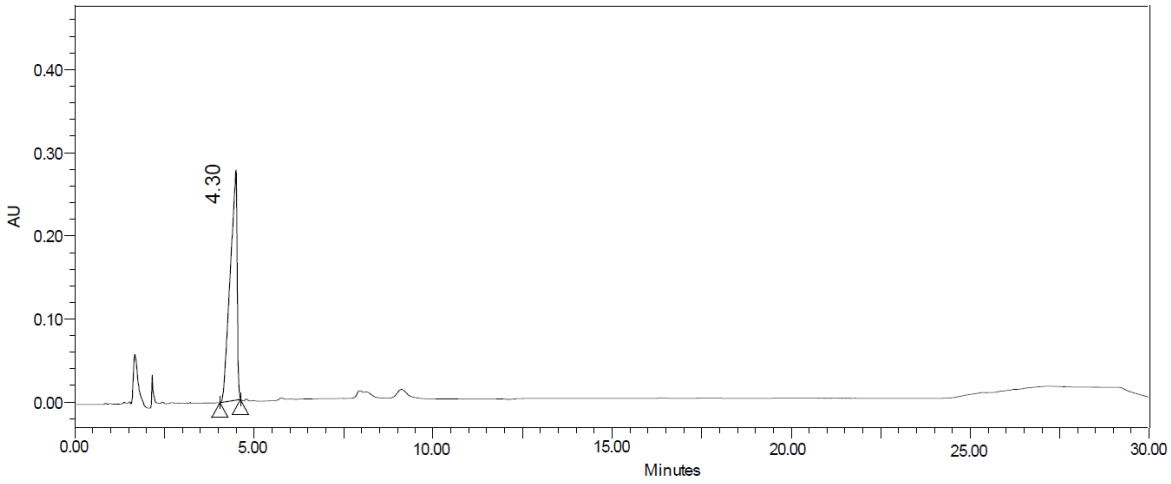


Fig 5.3.4 (a): HPLC chromatograms showing bile resistance of XOS with 1 % at 0h (1)

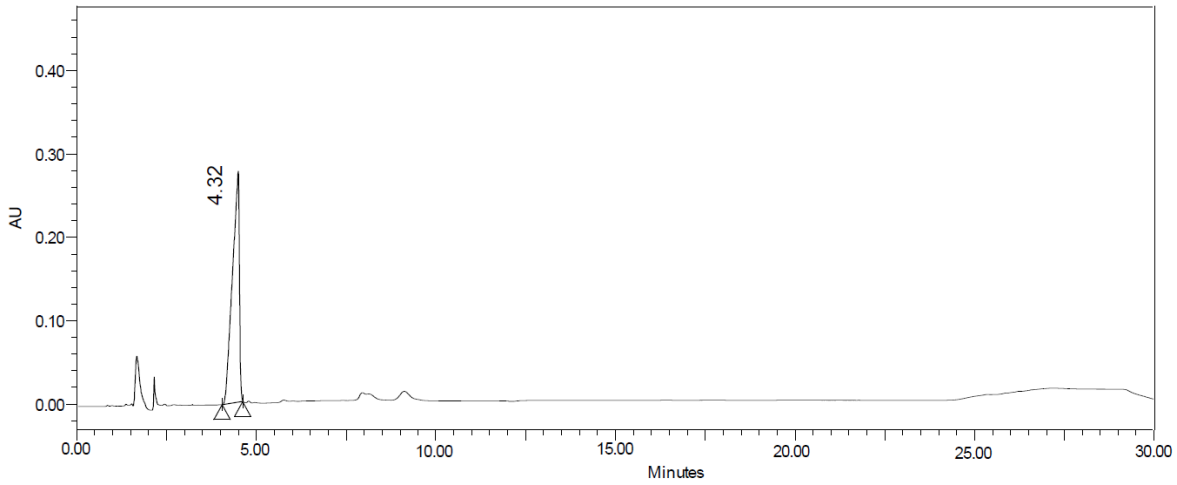


Fig 5.3.4 (b): HPLC chromatograms showing bile resistance of XOS with 1 % at 0h (2)

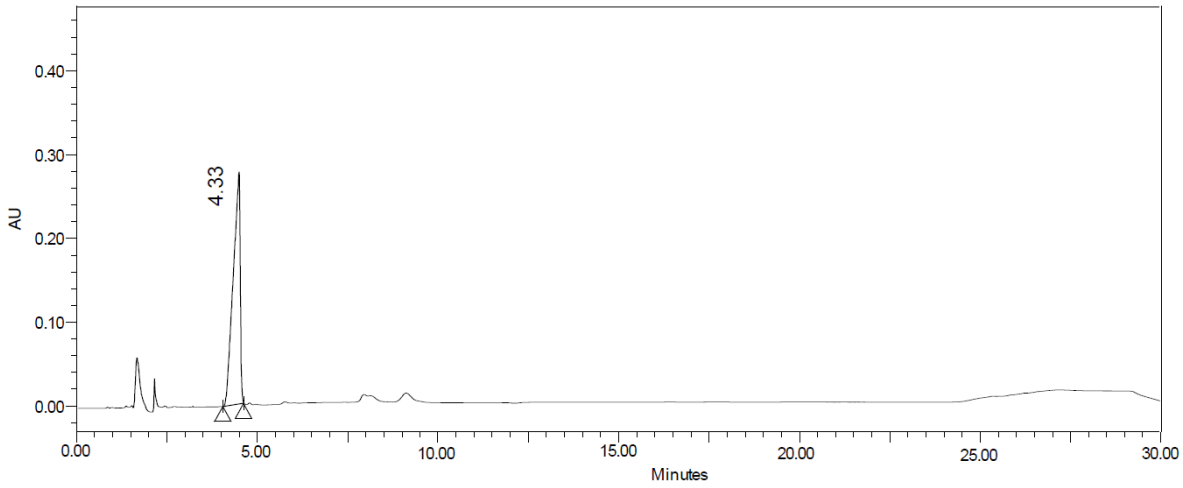


Fig 5.3.5 (a): HPLC chromatograms showing bile resistance of XOS with 1 % at 1.5h (1)

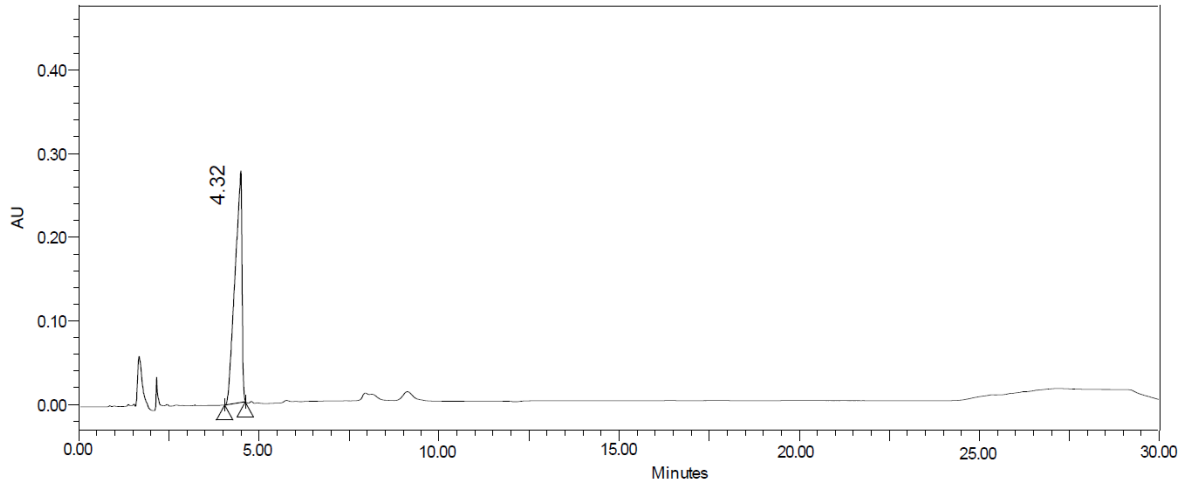


Fig 5.3.5 (b): HPLC chromatograms showing bile resistance of XOS with 1 % at 1.5h (2)

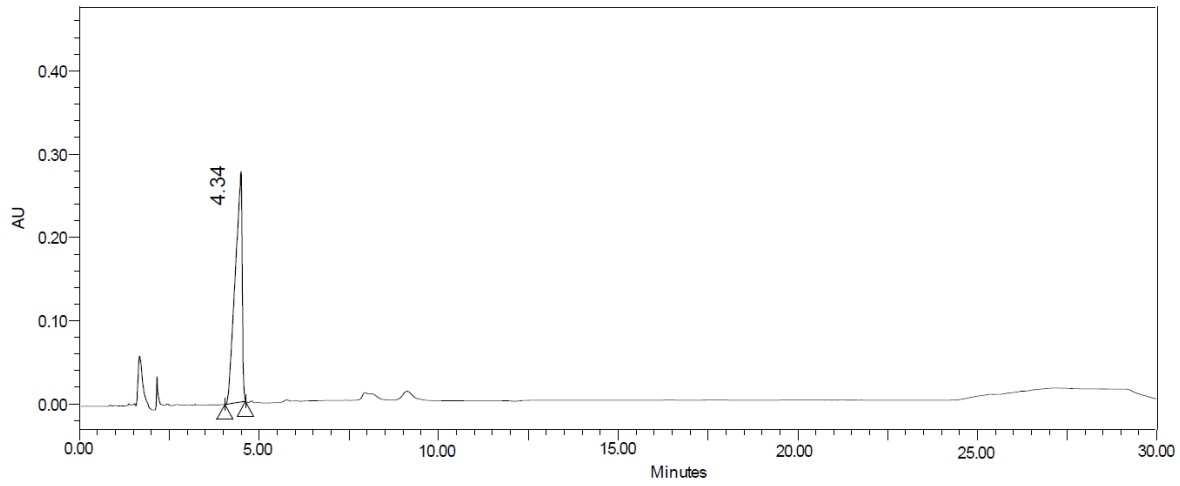


Fig 5.3.6 (a): HPLC chromatograms showing bile resistance of XOS with 1 % at 3h (1)

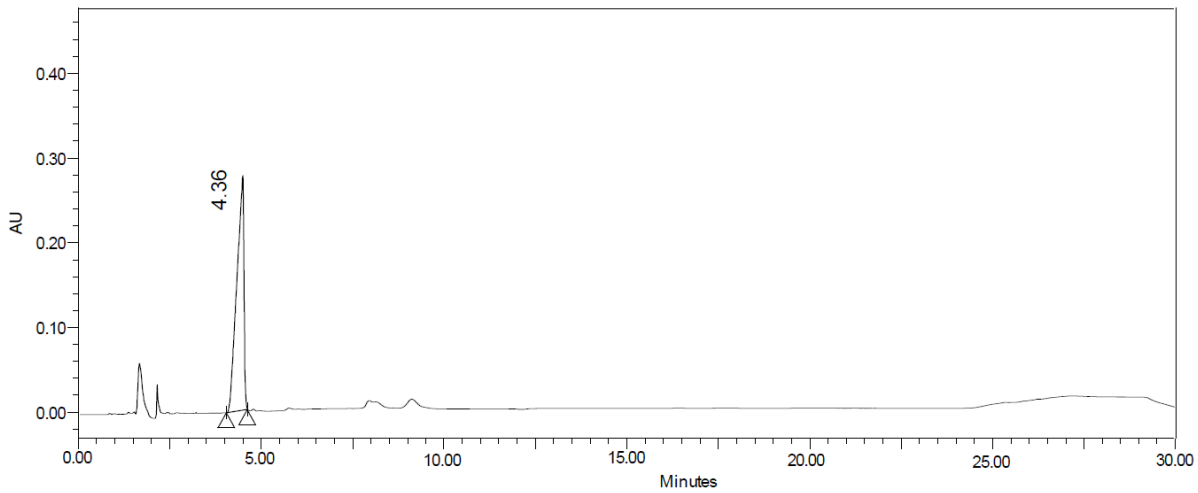


Fig 5.3.6 (b): HPLC chromatograms showing bile resistance of XOS with 1 % at 3h (2)

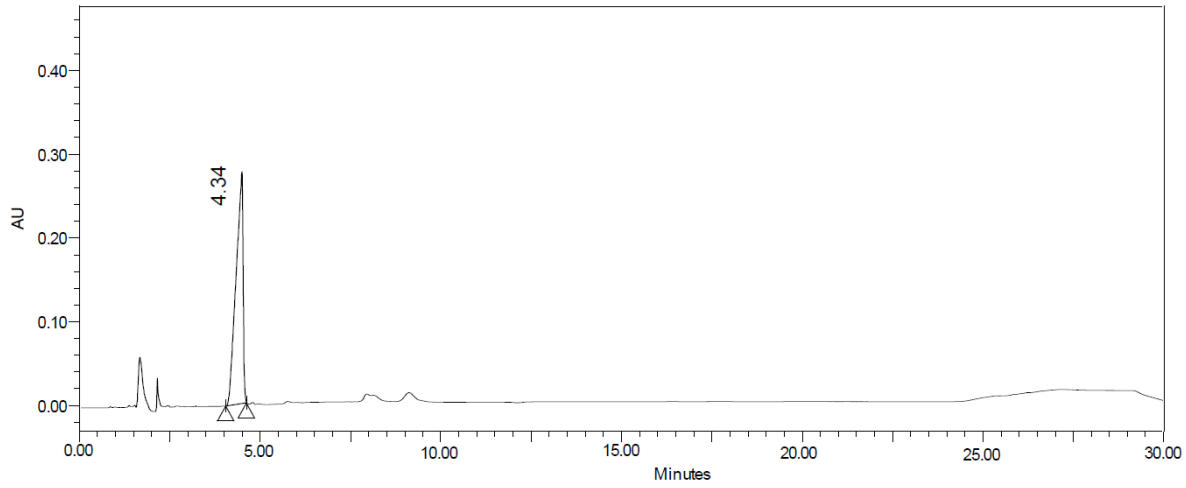


Fig 5.3.7 (a): HPLC chromatograms showing bile resistance of XOS with 1.5 % at 0h (1)

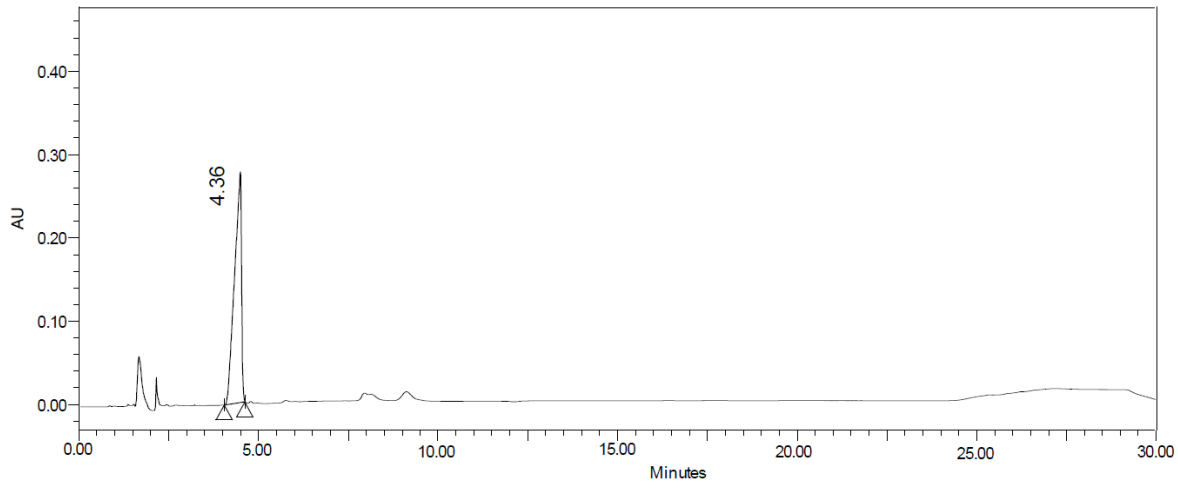


Fig 5.3.7 (b): HPLC chromatograms showing bile resistance of XOS with 1.5 % at 0h (2)

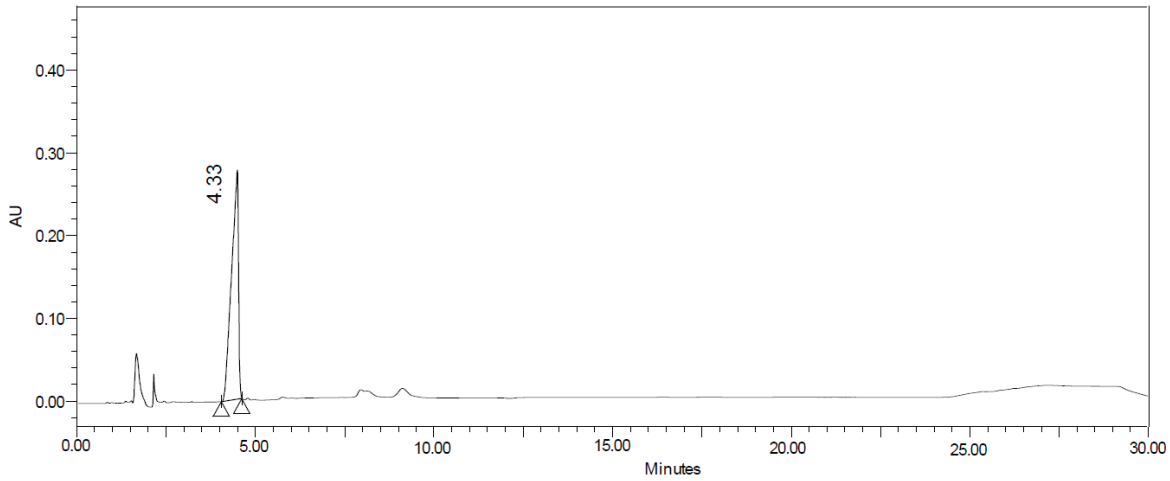


Fig 5.3.8 (a): HPLC chromatograms showing bile resistance of XOS with 1.5 % at 1.5h (1)

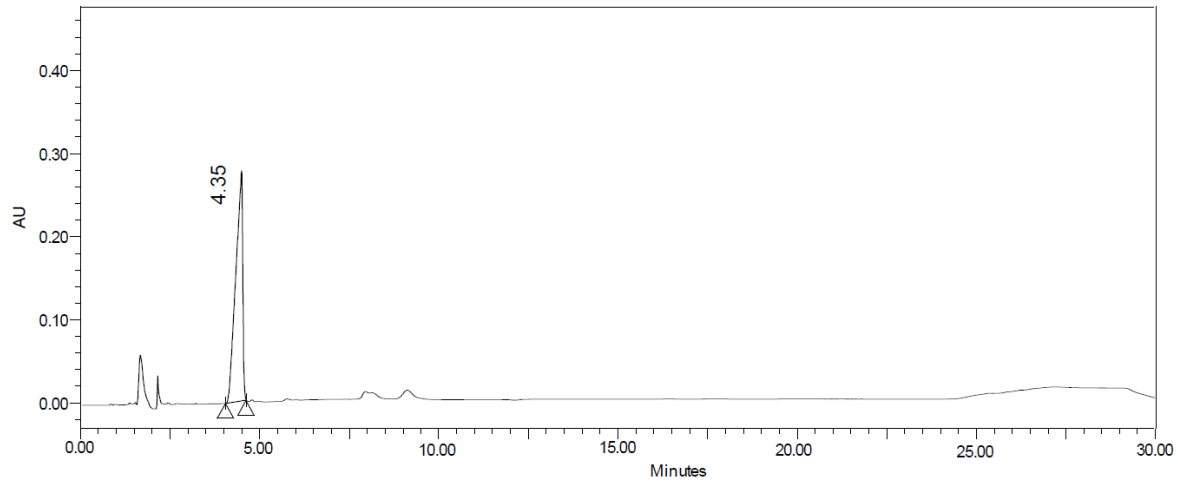


Fig5.3.8 (b): HPLC chromatograms showing bile resistance of XOS with 1.5 % at 1.5h (2)

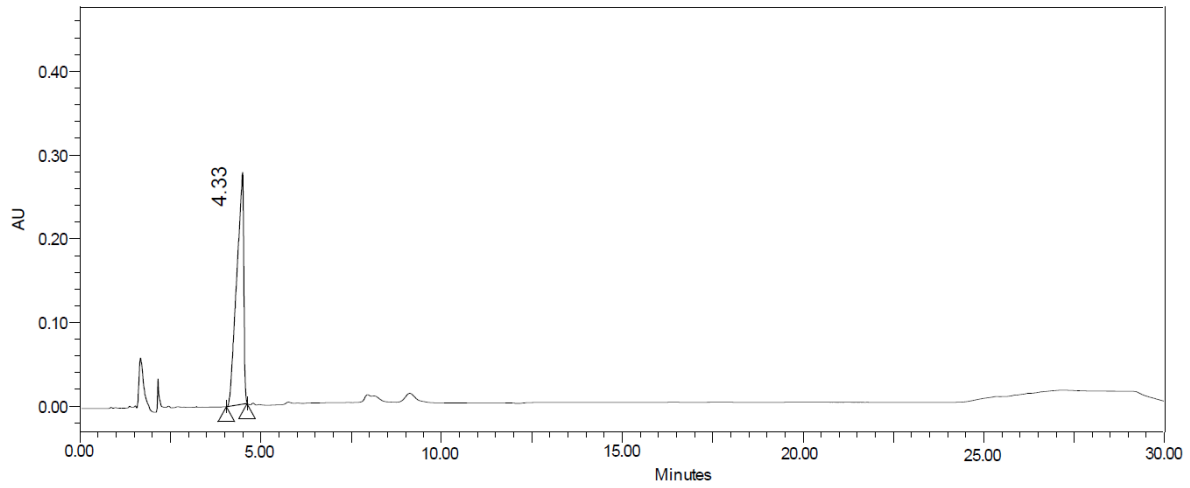


Fig 5.3.9 (a): HPLC chromatograms showing bile resistance of XOS with 1.5 % at 3h (1)

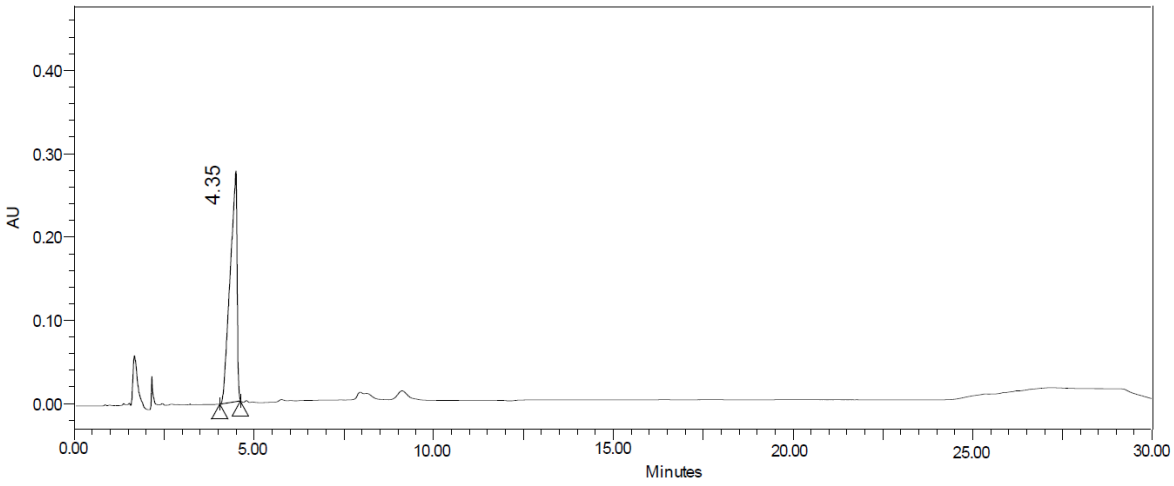


Fig 5.3.9 (b): HPLC chromatograms showing bile resistance of XOS with 1.5 % at 3h (2)

Fig 5.4.1 (a) – 5.4.11 (b): HPLC chromatograms in duplicates showing acid tolerance of XOS with 1.5pH, 2pH and 3pH at 0h, 1.5h and 3h.

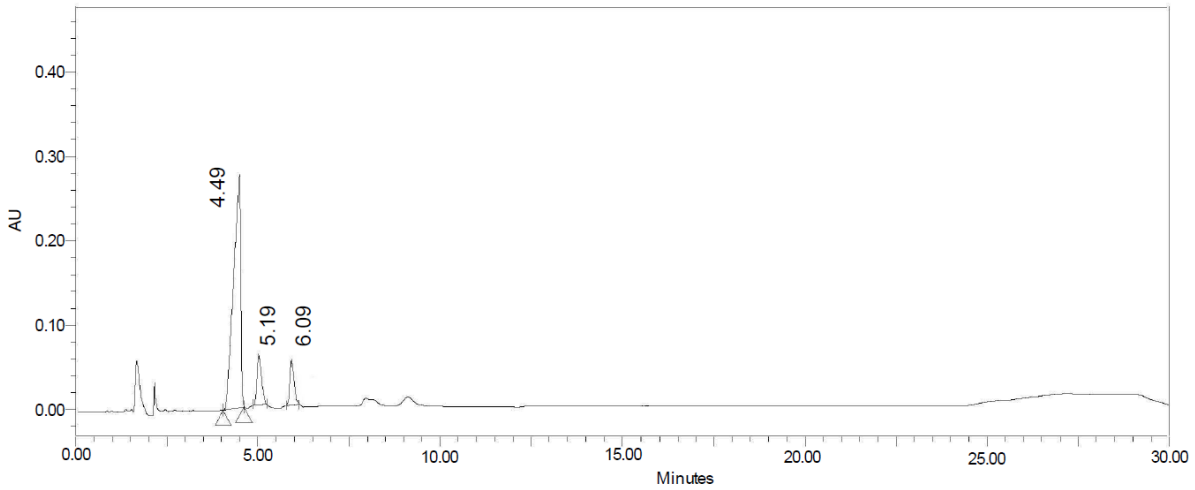


Fig 5.4.1 (a): HPLC chromatograms showing acid tolerance of XOS with 1.5pH at 0h (1)

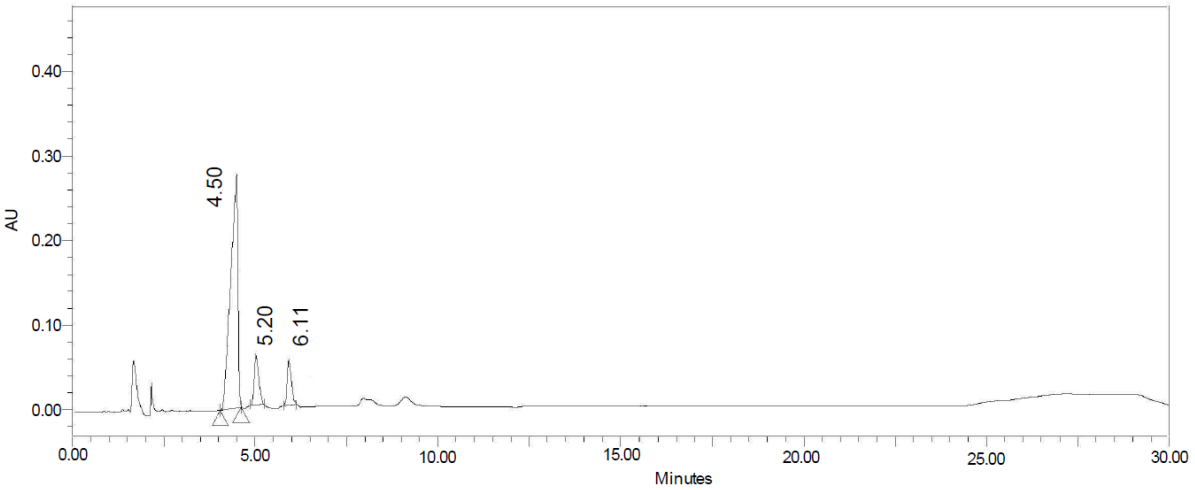


Fig 5.4.2 (a): HPLC chromatograms showing acid tolerance of XOS with 1.5pH at 0h (2)

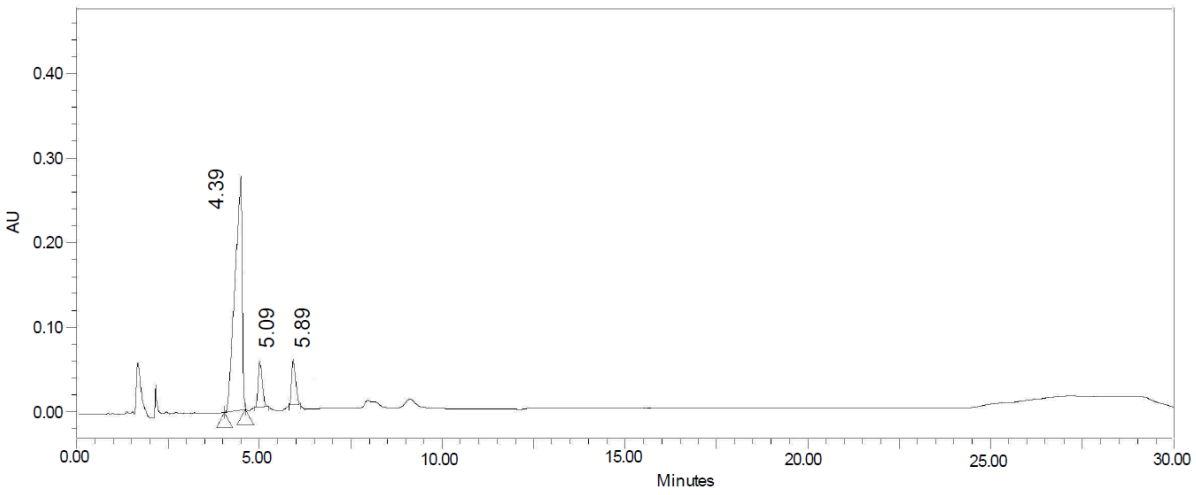


Fig 5.4.3 (a): HPLC chromatograms showing acid tolerance of XOS with 1.5pH at 1.5h (1)

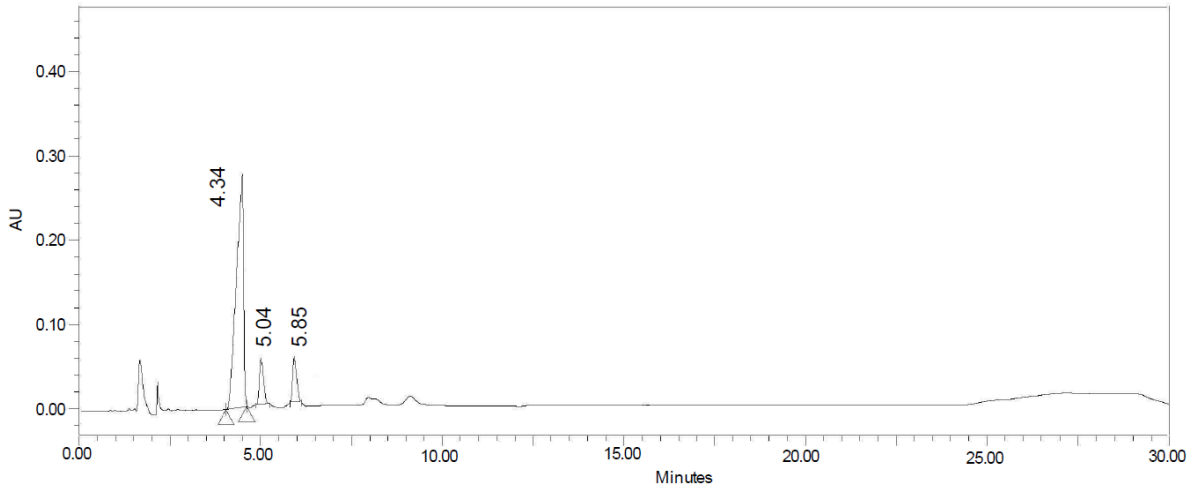


Fig 5.4.3 (b): HPLC chromatograms showing acid tolerance of XOS with 1.5pH at 1.5h (2)

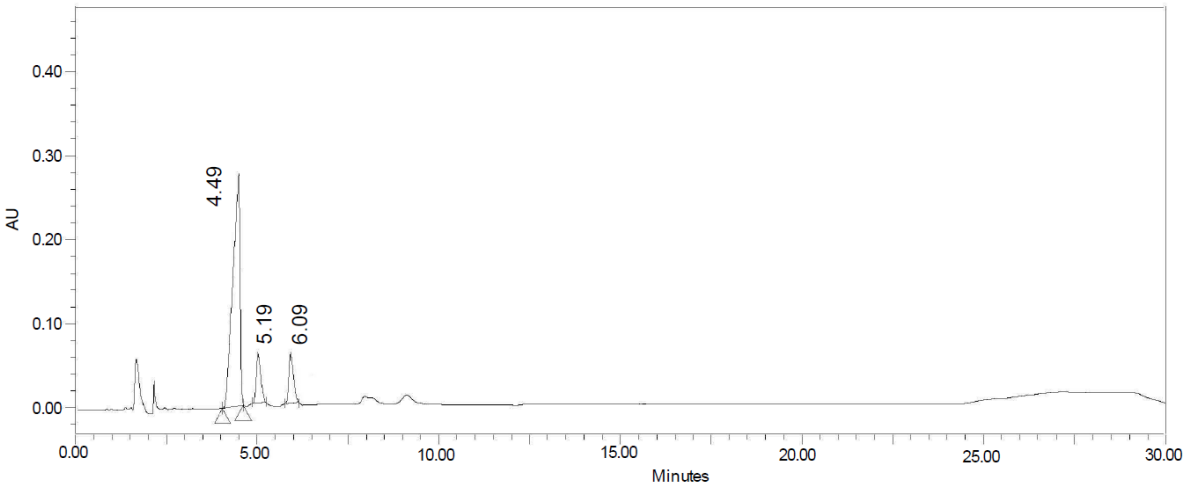


Fig 5.4.4 (a): HPLC chromatograms showing acid tolerance of XOS with 1.5pH at 3h (1)

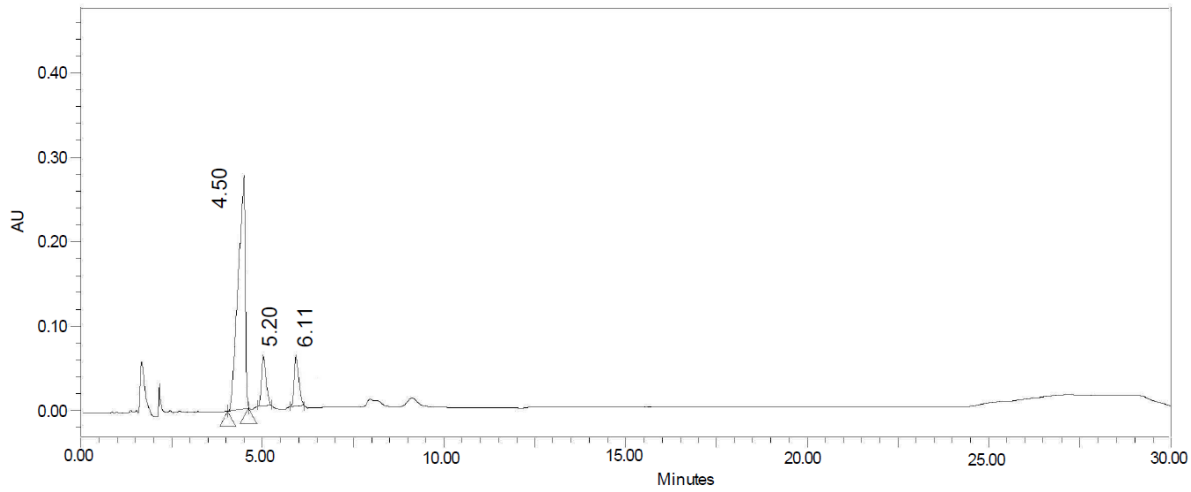


Fig 5.4.4 (b): HPLC chromatograms showing acid tolerance of XOS with 1.5pH at 3h (2)

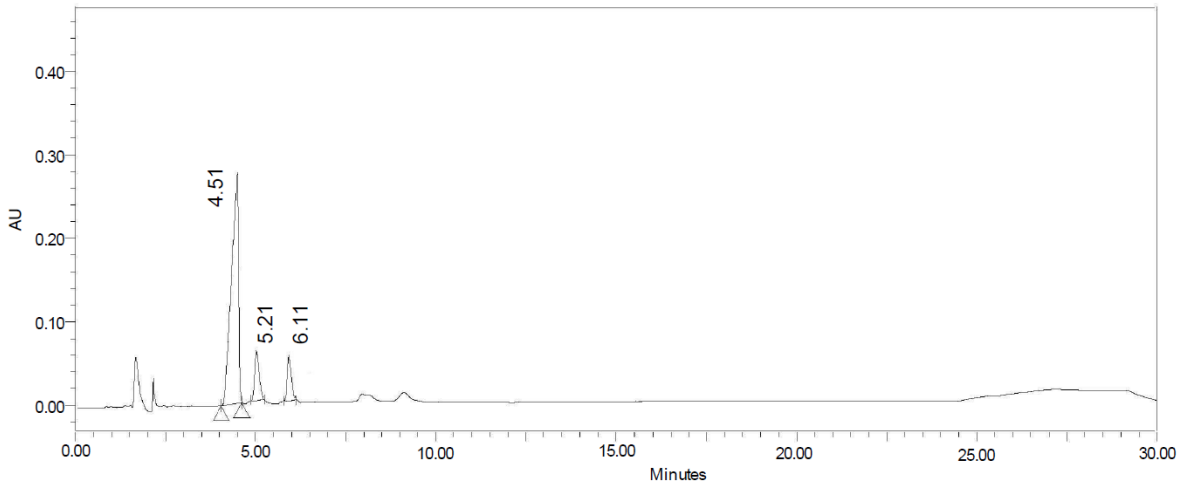


Fig 5.4.5 (a): HPLC chromatograms showing acid tolerance of XOS with 2pH at 0h (1)

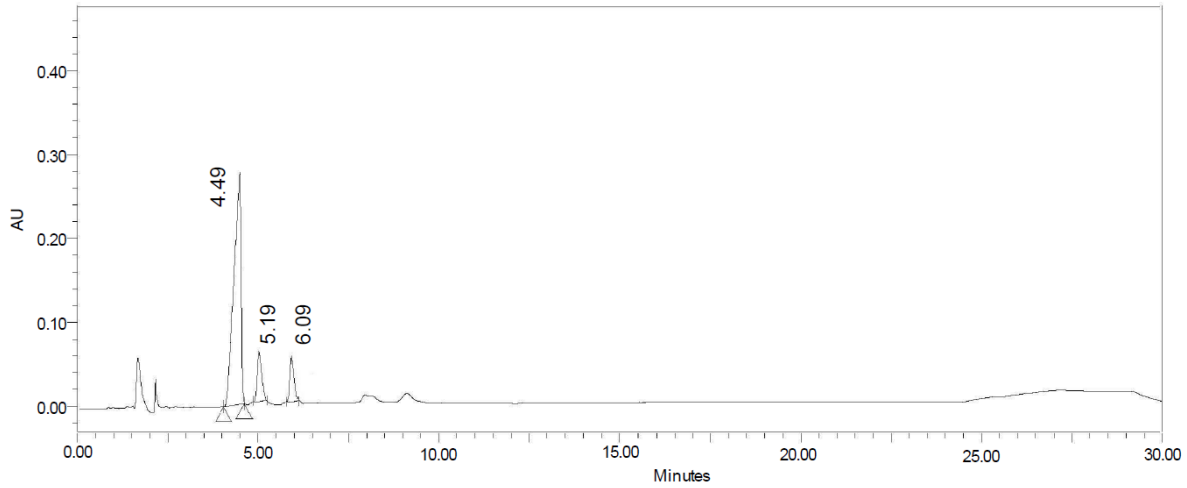


Fig 5.4.5 (b): HPLC chromatograms showing acid tolerance of XOS with 2pH at 0h (2)

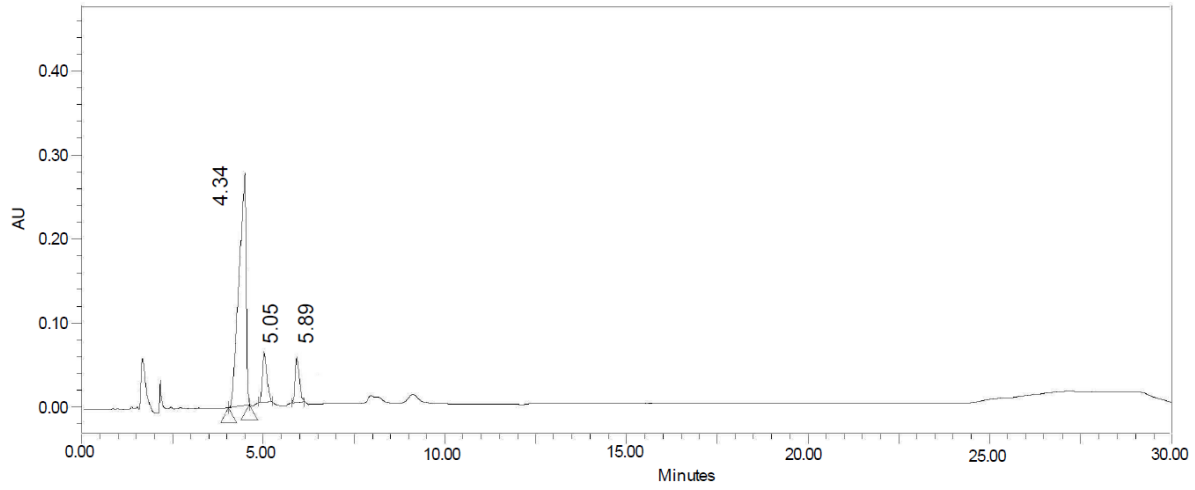


Fig 5.4.7 (a): HPLC chromatograms showing acid tolerance of XOS with 2pH at 1.5h (1)

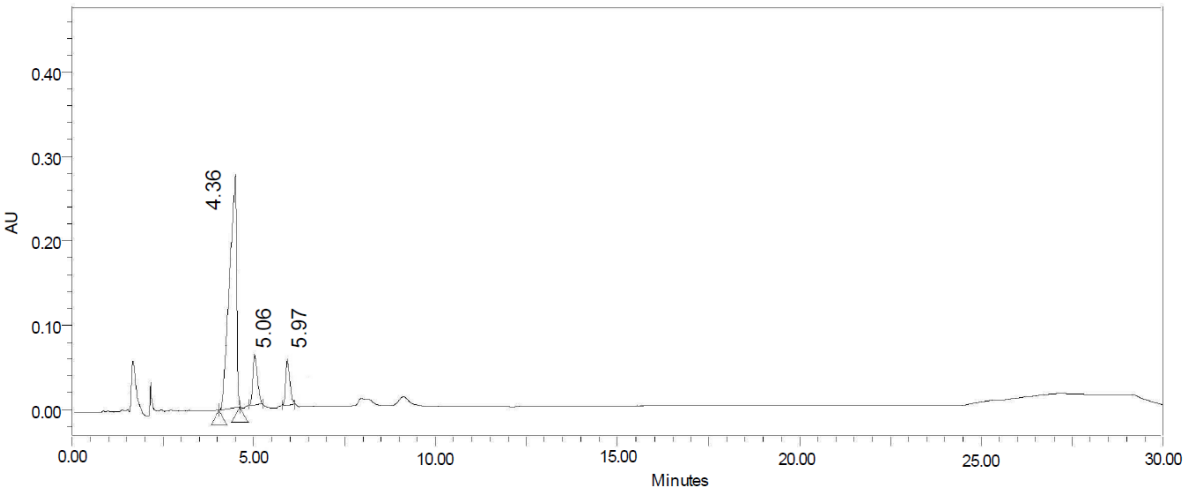


Fig 5.4.7 (b): HPLC chromatograms showing acid tolerance of XOS with 2pH at 1.5h (2)

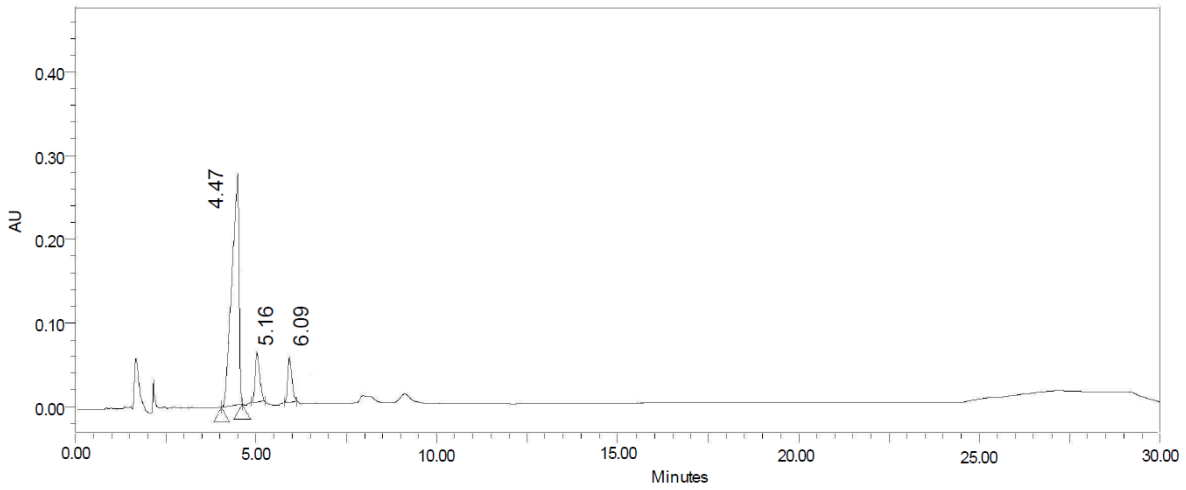


Fig 5.4.8 (a): HPLC chromatograms showing acid tolerance of XOS with 2pH at 3h (1)

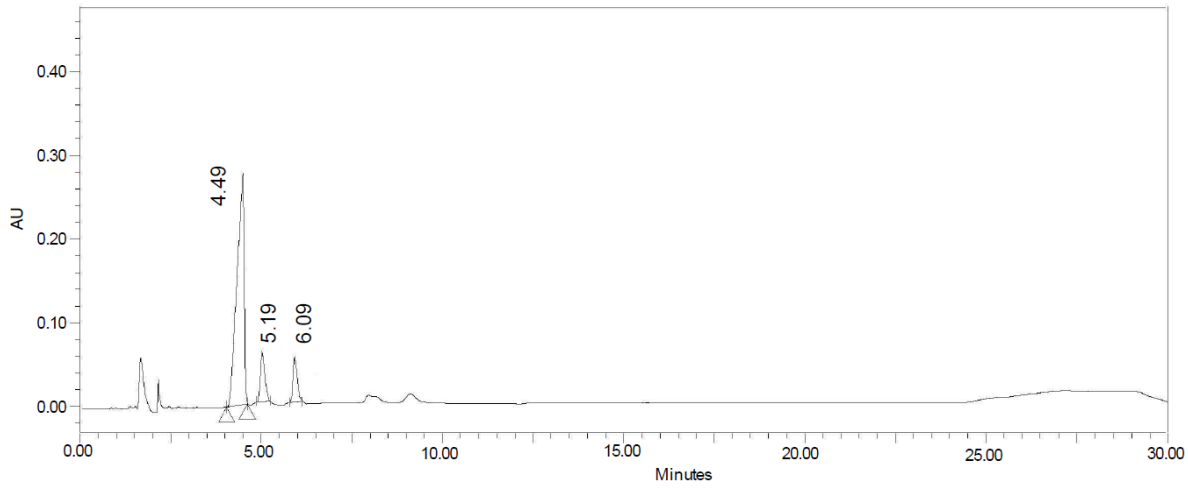


Fig 5.4.8 (b): HPLC chromatograms showing acid tolerance of XOS with 2pH at 3h (2)

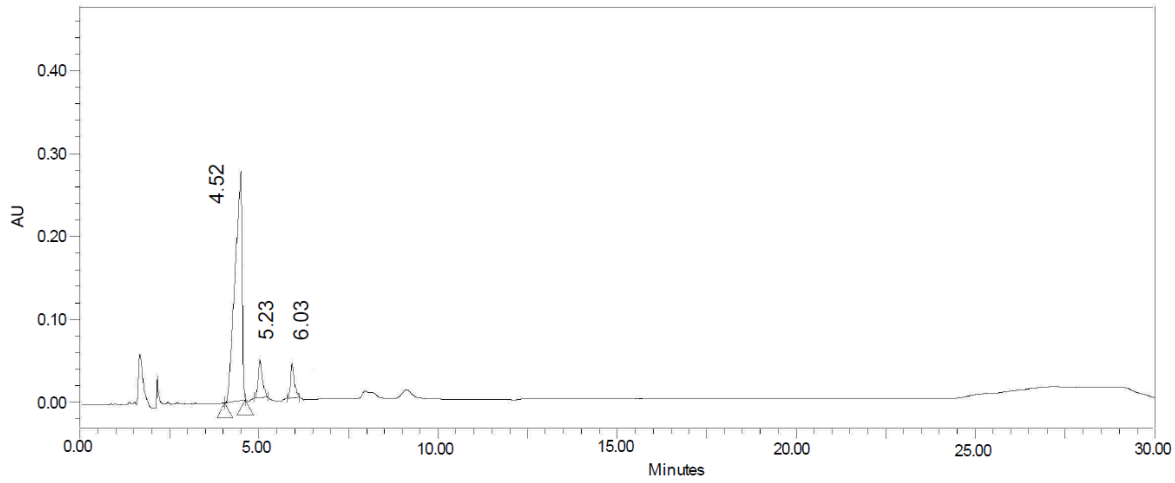


Fig 5.4.9 (a): HPLC chromatograms showing acid tolerance of XOS with 3pH at 0h (1)

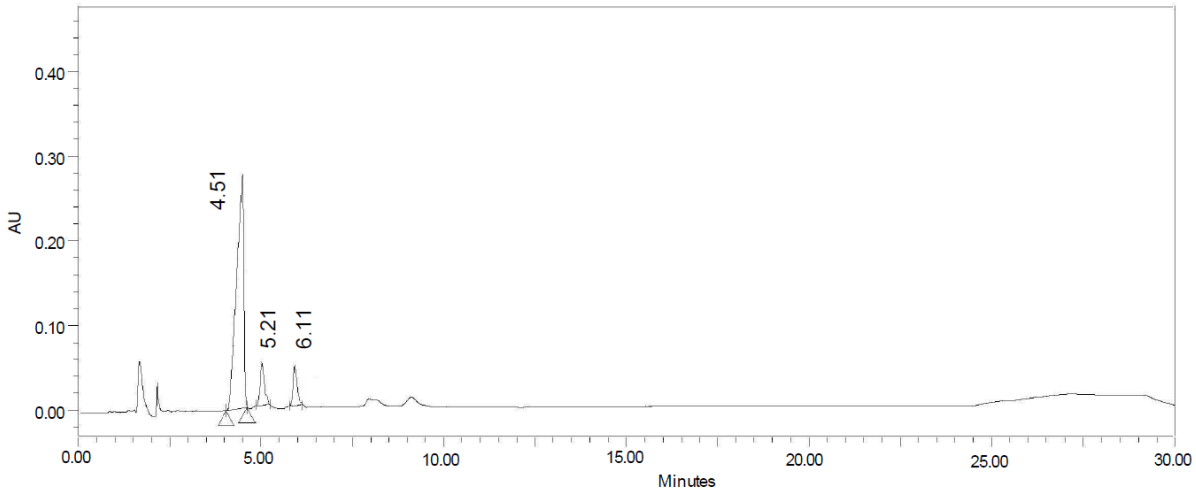


Fig 5.4.9 (b): HPLC chromatograms showing acid tolerance of XOS with 3pH at 0h (2)

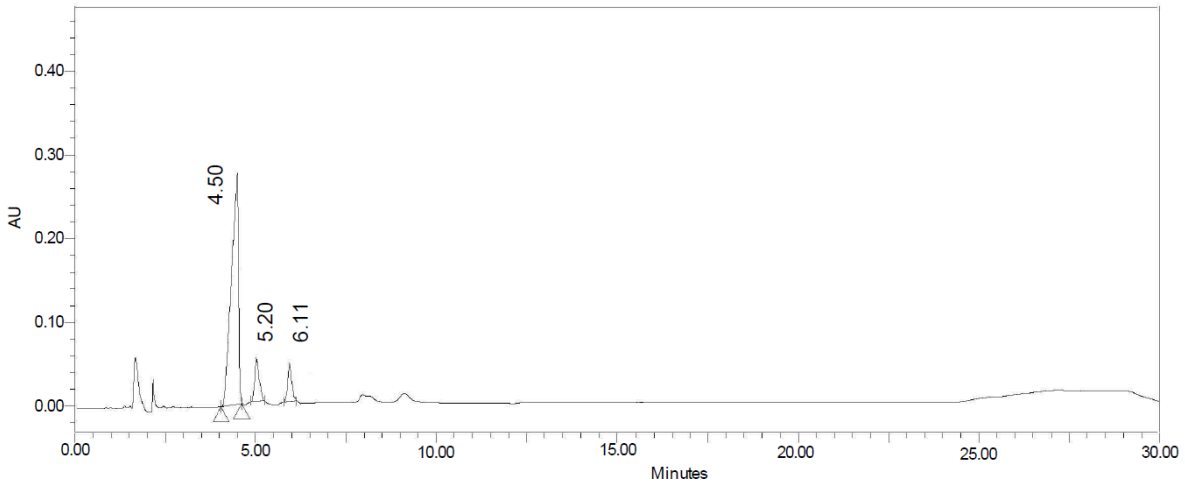


Fig 5.4.10 (a): HPLC chromatograms showing acid tolerance of XOS with 3pH at 1.5h (1)

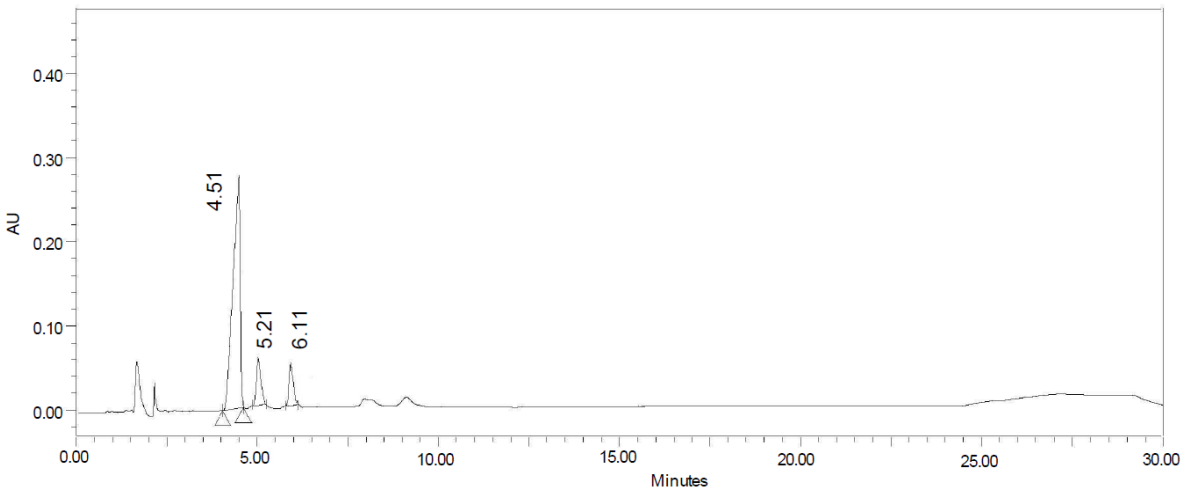


Fig 5.4.10 (b): HPLC chromatograms showing acid tolerance of XOS with 3pH at 1.5h (2)

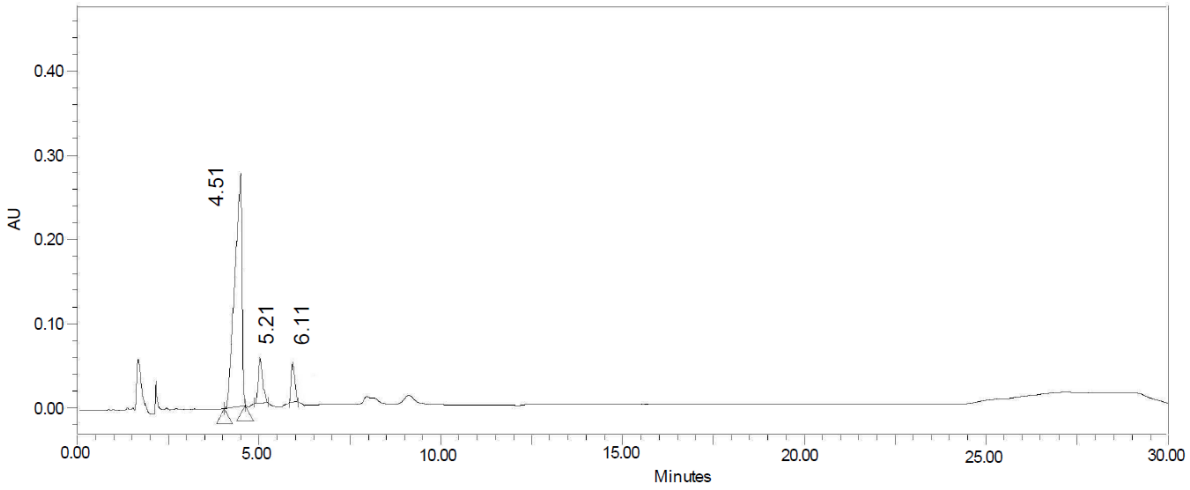


Fig 5.4.11 (a): HPLC chromatograms showing acid tolerance of XOS with 3pH at 3h (1)

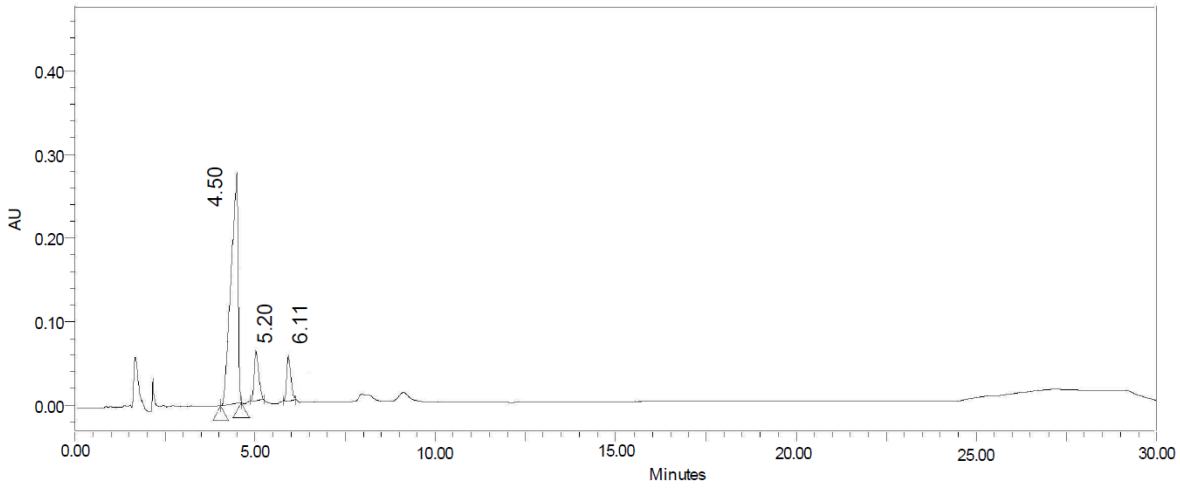
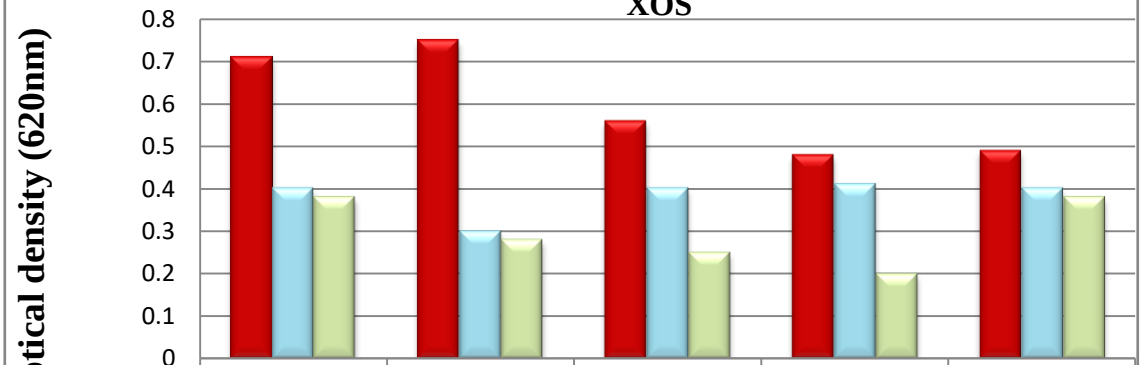


Fig 5.4.11 (b): HPLC chromatograms showing acid tolerance of XOS with 3pH at 3h (2)

5.6. Prebiotic effect of XOS on the growth of *L. plantarum, B. adolescentis* and *E. coli*

As seen in figure 5.5, the growth of *Lactobacillus plantarum (LP)* and *Bifidobacterium adolescentis (BA)* were higher at 0.5%, 1% and 2% of XOS addition. For *Escherichia coli (E.coli)* the growth gradually decreased as the concentration of XOS increased from 0.5% to 2%. Since 0.5%, 1% and 2% levels of XOS concentration gave better or almost equivalent growth of *Lactobacillus plantarum (LP), Bifidobacterium adolescentis (BA)* and reduced the growth of *Escherichia coli (E. coli).* Therefore, 0.5%, 1% and 2% levels of XOS concentration samples were chosen for production of short chain fatty acids (SCFA) and its analysis.

Fig. 5.5: Impact on the growth of *Lactobacillus plantarum, Bifidobacterium adolescentis* and *Escerichia coli* with XOS



	0.50%	1%	2%	3%	4%
O.D. (LP)	0.71	0.75	0.56	0.48	0.49
O.D.(BA)	0.4	0.3	0.4	0.41	0.4
O.D.(EC)	0.38	0.28	0.25	0.2	0.38

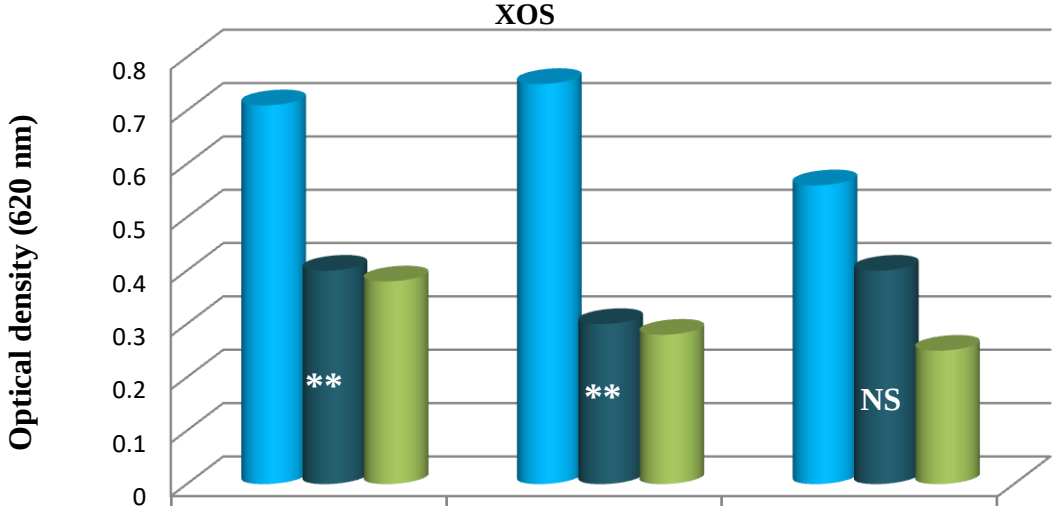
Table 5.5 shows mean growth in O.D. and SD of *Lactobacillus plantarum, Bifidobacterium adolescentis* and *Escherichia coli* with 0.5%, 1% and 2% levels of XOS.

Table 5.5:Mean growth and SD of *Lactobacillus plantarum, Bifidobacterium adolescentis* and *Escherichia coli* with 0.5%, 1% and 2% levels of XOS

XOS (%)	*Lactobacillus plantarum(LP)* Mean±SD	*Bifidobacterium adolescentis(BA)* Mean±SD	*Escherichia coli(EC)* Mean±SD
0.5	0.71 ± 0.24	0.4 ± 0.09	0.38 ± 0.04
1	0.75 ± 0.16	0.3 ± 0.00	0.28 ± 0.04
2	0.56 ± 0.31	0.4 ± 0.07	0.25 ± 0.03
3	0.48 ± 0.07	0.41 ± 0.05	0.2 ± 0.03
4	0.49 ± 0.13	0.40 ± 0.03	0.38 ± 0.05

Figure 5.6, shows mean growth of *Lactobacillus plantarum* was more with 0.5% and 1% XOS concentration at p≤0.01, growth of *Bifidobacterium adolescentis* was seen to be same with 0.5% and 2% XOS concentration at p≤0.01 and growth of *Escherichia coli* was the least with 1% XOS.

Fig: 5.6. Mean growth of *Lactobacillus plantarum, Bifidobacterium adolescentis* and *Escherichia coli* with 0.5%, 1% and 2% levels of XOS



	XOS 0.5%	XOS 1%	XOS 2%
O.D. (LP)	0.71	0.75	0.56
O.D.(BA)	0.4	0.3	0.4
O.D.(EC)	0.38	0.28	0.25

Significant at *p≤0.05, **p≤0.01, *** p≤0.001, NS- non significant

Table 5.6 shows mean growth in O.D. and SD of *Lactobacillus plantarum, Bifidobacterium adolescentis* and *Escherichia coli* with 0.5%, 1% and 2% levels of XOS.

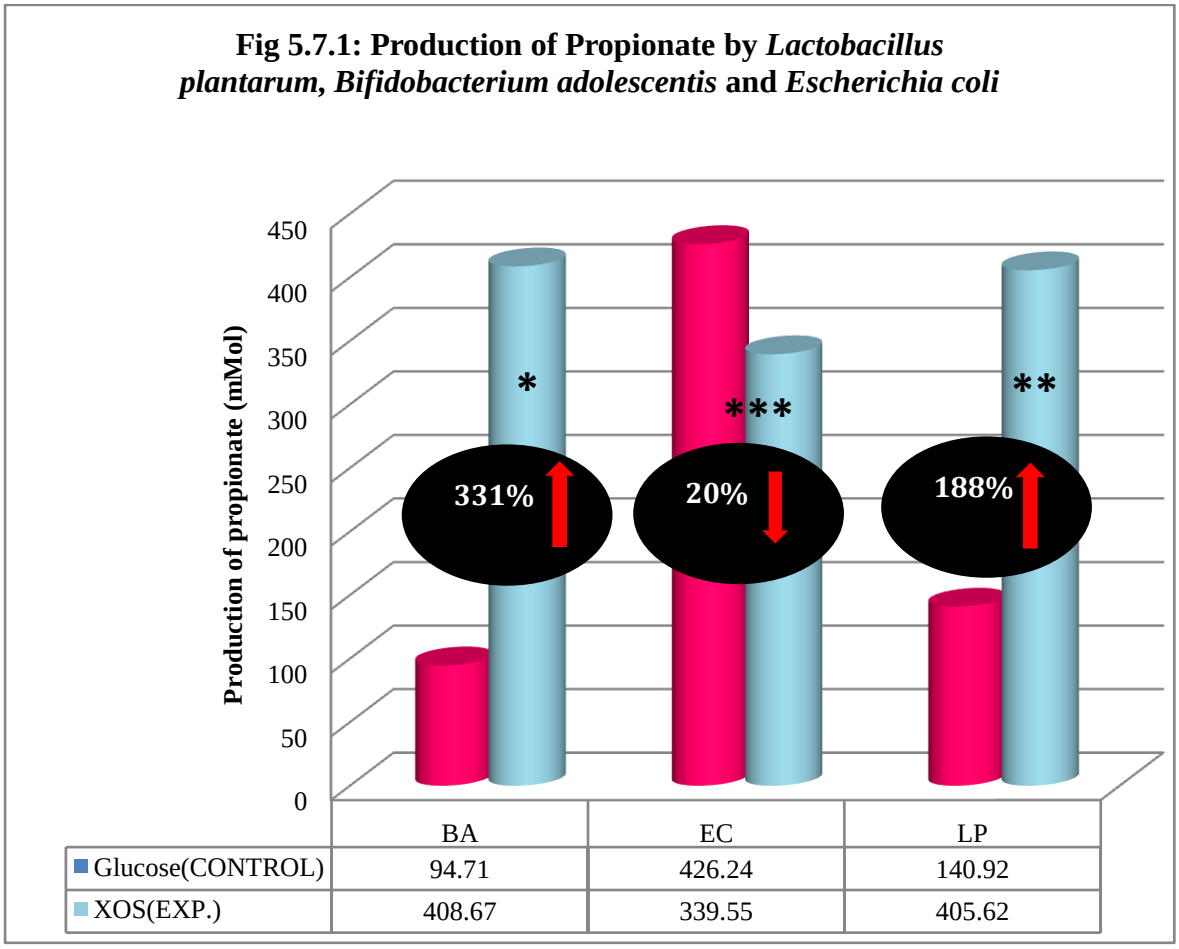
Table 5.6:Mean growth and SD of *Lactobacillus plantarum, Bifidobacterium adolescentis* and *Escherichia coli* with 0.5%, 1% and 2% levels of XOS

XOS (%)	*Lactobacillus plantarum(LP)* Mean±SD	*Bifidobacterium adolescentis(BA)* Mean±SD	*Escherichia coli(EC)* Mean±SD
0.5	0.71±0.24	0.4 ± 0.09	0.38 ± 0.04
1	0.75±0.16	0.3 ± 0.00	0.28 ± 0.04
2	0.56±0.31	0.4 ± 0.07	0.25 ± 0.03

5.7. SCFA production analysis during fermentation in vitro

Figure 5.7.1, reveals that *Bifidobacterium adolescentis* produced 408.6 mMol(↑331%) propionate on its exposure to XOS. *Lactobacillus plantarum* produced 405.62mMol (↑188%) propionate on its exposure to XOS. When *Escherichia coli* were exposed to XOS production of propionate reduced 339.55 mMol (↓20%).

Fig 5.7.1: Production of Propionate by *Lactobacillus plantarum, Bifidobacterium adolescentis* and *Escherichia coli*

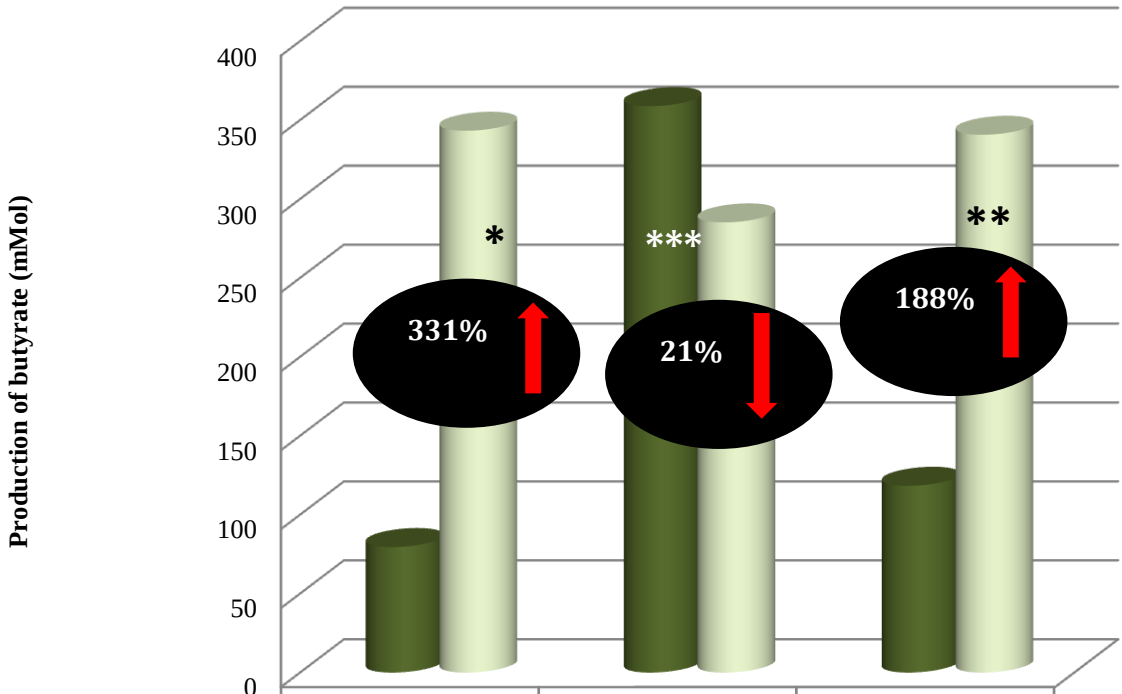


	BA	EC	LP
Glucose(CONTROL)	94.71	426.24	140.92
XOS(EXP.)	408.67	339.55	405.62

Significant at *p≤0.05, **p≤0.01, *** p≤0.001, NS- non significant

Figure 5.7.2, reveals that *Bifidobacterium adolescentis* produced 343.28 mMol(↑331%) butyrate on its exposure to XOS. *Lactobacillus plantarum* produced 340.72 mMol (↑188%) of butyrate on its exposure to XOS. When *Escherichia coli* were exposed to XOS production of butyrate reduced 285.22 mMol (↓21%).

Fig 5.7.2: Production of Butyrate by *Lactobacillus plantarum, Bifidobacterium adolescentis* and *Escherichia coli*

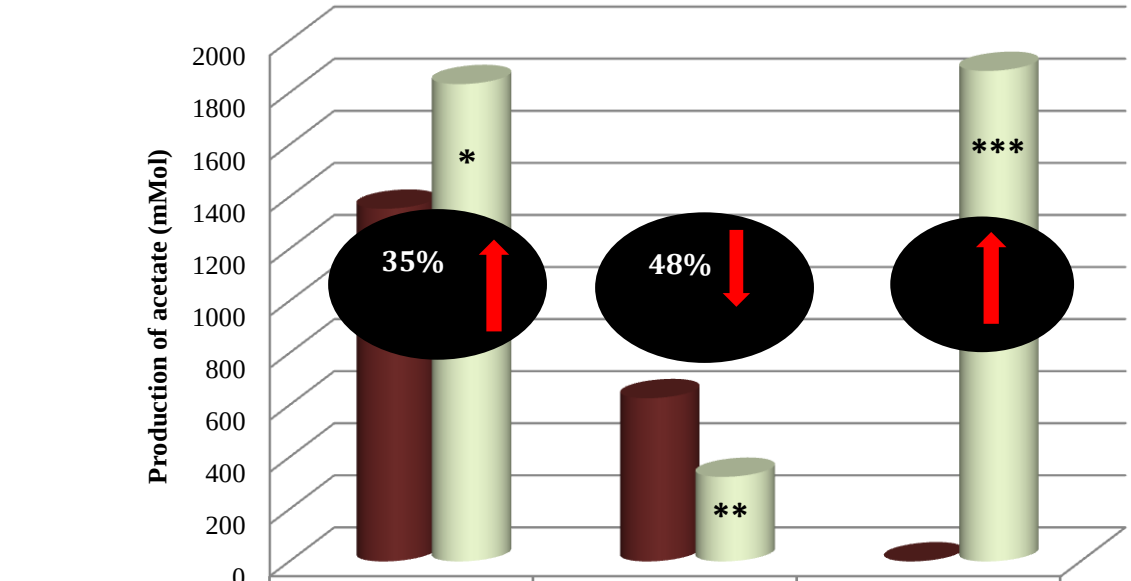


	BA	EC	LP
Glucose(CONTROL)	79.56	358.88	118.37
XOS(EXP.)	343.28	285.22	340.72

Significant at *p≤0.05, **p≤0.01, *** p≤0.001, NS- non significant

Figure 5.7.3, reveals that *Bifidobacterium adolescentis* produced 1833.27 mMol (↑35%) acetate on its exposure to XOS. *Lactobacillus plantarum* produced 1883.82 mMol of acetate on its exposure to XOS and produced 0 mMol when exposed to glucose. When *Escherichia coli* were exposed to XOS production of acetate reduced to 324.65 mMol (↓48%).

Fig 5.7.3 : Production of Acetate by *Lactobacillus plantarum, Bifidobacterium adolescentis* and *Escherichia coli*



	BA	EC	LP
Glucose(CONTROL)	1354.54	626.85	0
XOS(EXP.)	1833.27	324.65	1883.82

Significant at *p≤0.05, **p≤0.01, *** p≤0.001, NS- non significant

Table 5.7 reveals mean and SD values of acetate, butyrate and propionate produced by *Lactobacillus plantarum, Bifidobacterium acidophilus* and *Escherichia coli* on its exposure to XOS and glucose being used as control group.

Table 5.7: Mean and SD values of acetate, butyrate and propionate produced by *Lactobacillus plantarum, Bifidobacterium acidophilus* and *Escherichia coli* on its exposure to XOS and glucose being used as control group.

	Acetate (mMol) **Mean ± SD**	**Butyrate (mMol)** **Mean ± SD**	**Propionate (mMol)** **Mean ± SD**
Lactobacillus plantarum + Glucose (LPG)	0	118.37 ± 7.84	140.92 ± 9.34
Lactobacillus plantarum + XOS (LPX)	1883.82 ± 115.23	340.72 ± 29.73	405.62 ± 35.39
Percent increase/decrease (↑/↓)	**-**	**↑ 188**	**↑ 188**
Bifidobacterium acidophilus + Glucose (BAG)	1354.54 ± 116.94	79.56 ± 93.12	94.71± 110.86
Bifidobacterium acidophilus + XOS (BAX)	1833.27 ± 0.00	343.28 ± 17.39	408.67 ± 20.70
Percent increase/decrease (↑/↓)	**↑ 35**	**↑ 331**	**↑ 331**
Escherichia coli+ Glucose (ECG)	636.85 ± 302.30	358.88 ± 0.00	427.24 ± 0.00
Escherichia coli+ XOS (ECX)	324.65 ± 3.48	285.22 ± 0.00	339.55 ± 0.00
Percent increase/decrease (↑/↓)	**↓ 48**	**↓ 21**	**↓ 20**

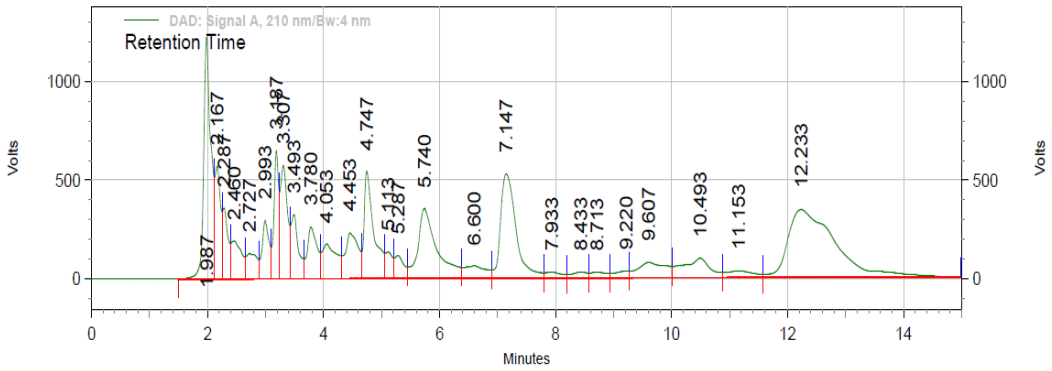


Fig 5.8.1 (a): HPLC chromatogram of *Bifidobacterium adolescentis* + XOS (BAX1) producing acetate, butyrate and propionate

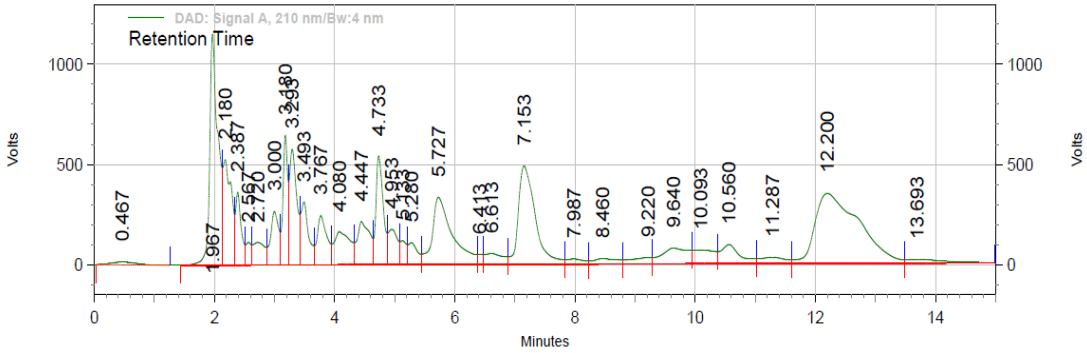


Fig. 5.8.1 (b): HPLC chromatogram of *Bifidobacterium adolescentis* + XOS (BAX2) producing acetate, butyrate and propionate

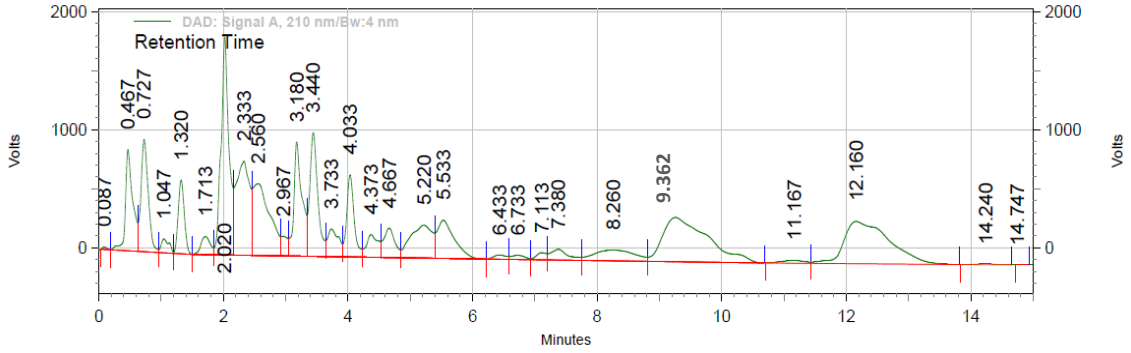


Fig. 5.8.2 (a): HPLC chromatogram of *Bifidobacterium adolescentis* + Glucose (BAG1) producing acetate, butyrate and propionate

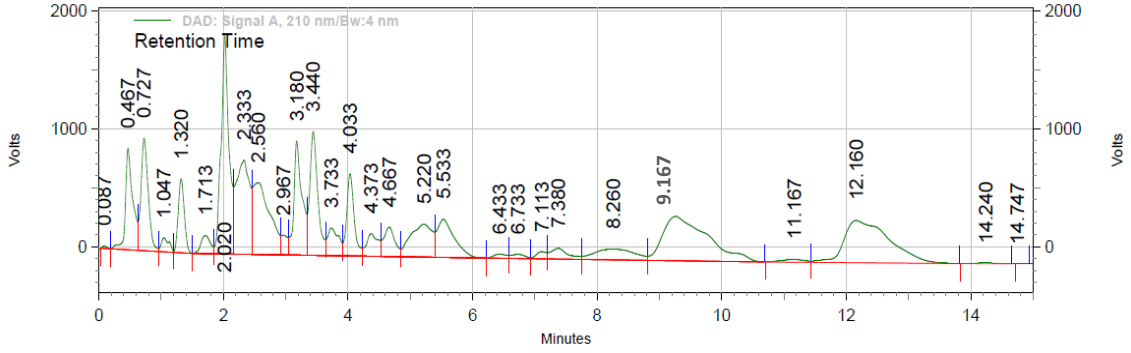


Fig. 5.8.2 (b): HPLC chromatogram of *Bifidobacterium adolescentis* + Glucose (BAG2) producing acetate, butyrate and propionate

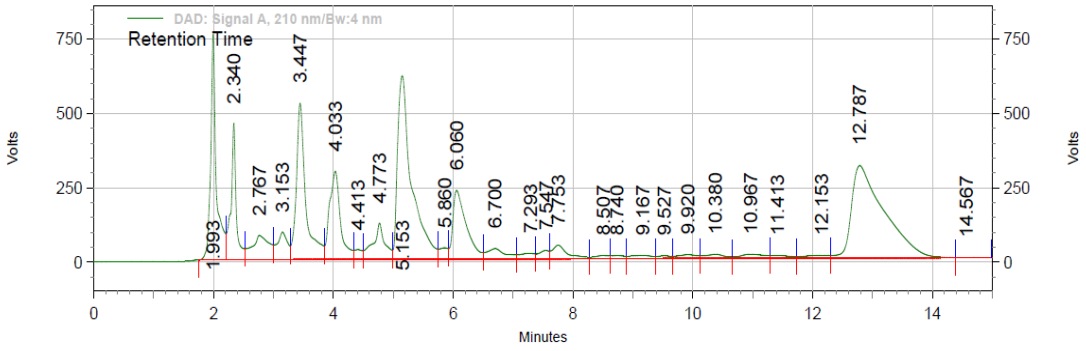


Fig. 5.8.3 (a): HPLC chromatogram of *Escherichia coli* + Glucose (ECG1) producing acetate, butyrate and propionate

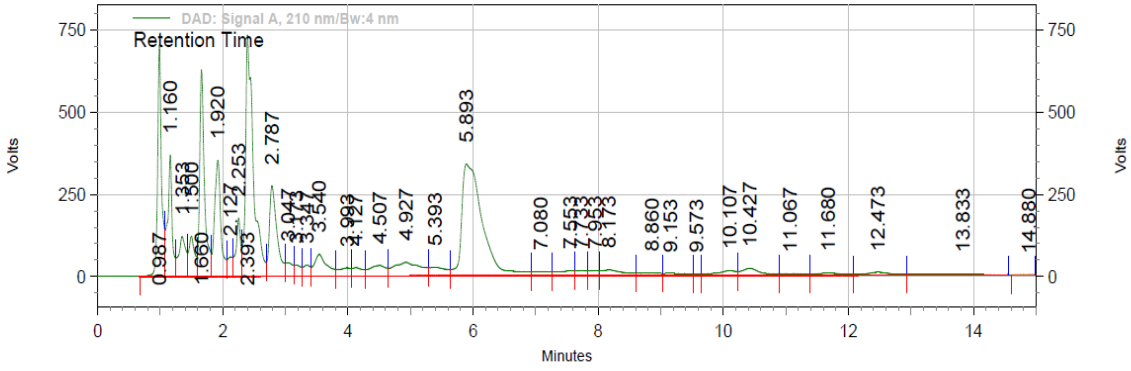


Fig. 5.8.3 (b): HPLC chromatogram of *Escherichia coli* + Glucose (ECG2) producing acetate, butyrate and propionate

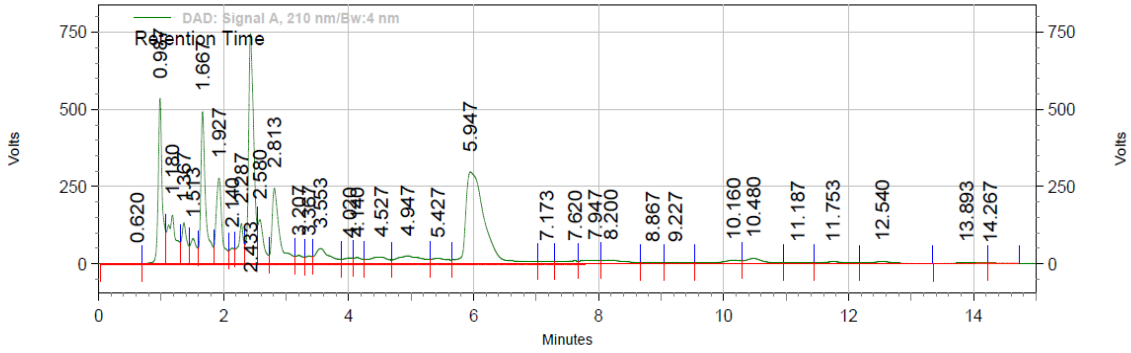


Fig. 5.8.4 (a): HPLC chromatogram of *Escherichia coli* + XOS (ECX1) producing acetate, butyrate and propionate

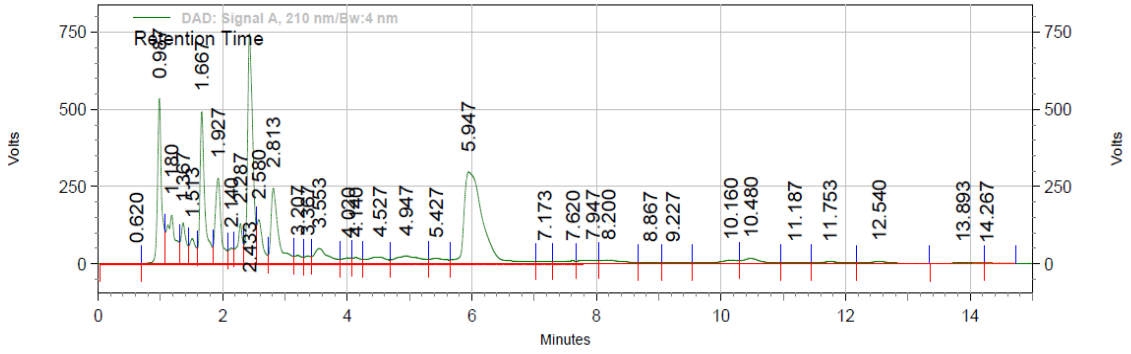


Fig. 5.8.4 (b): HPLC chromatogram of *Escherichia coli* + XOS (ECX2) producing acetate, butyrate and propionate

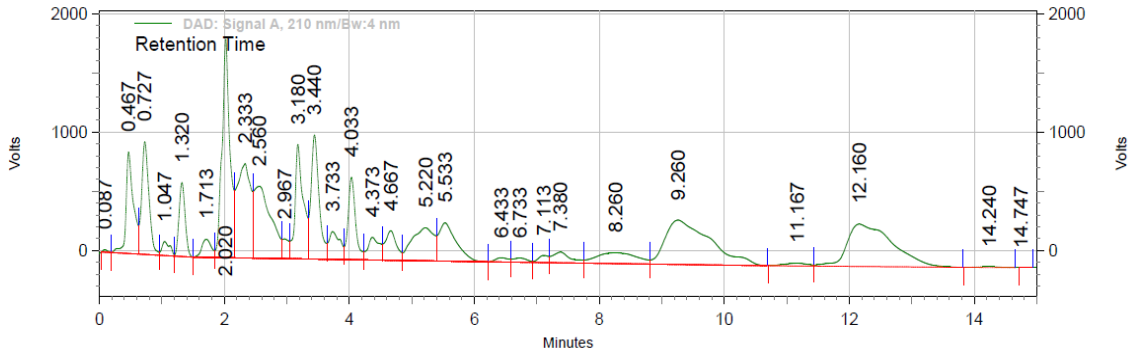


Fig. 5.8.5 (a): HPLC chromatogram of *Lactobacillus plantarum* + Glucose (LPG1) producing acetate, butyrate and propionate

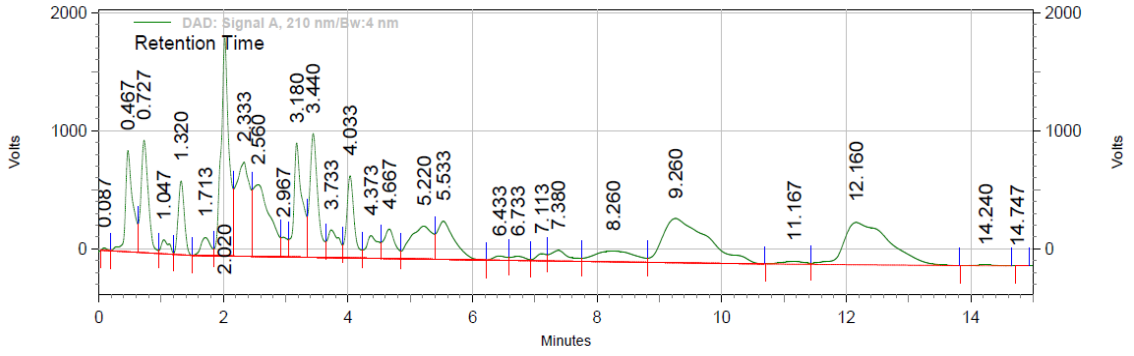


Fig. 5.8.5 (b): HPLC chromatogram of *Lactobacillus plantarum* + Glucose (LPG2) producing acetate, butyrate and propionate

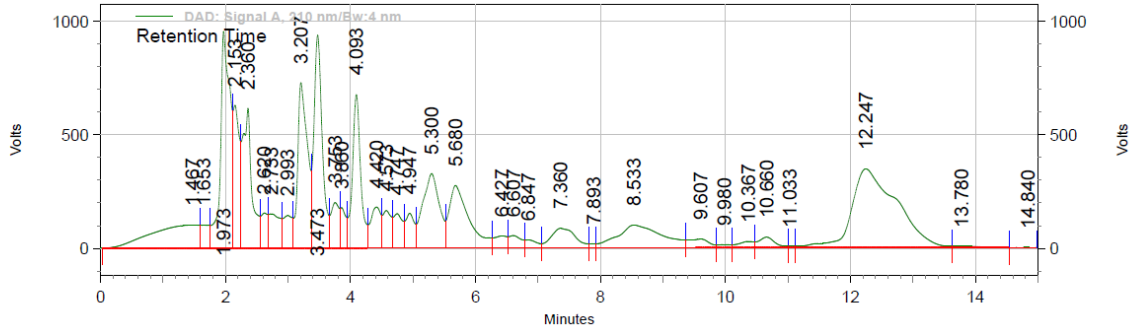


Fig. 5.8.6 (a): HPLC chromatogram of *Lactobacillus plantarum* + XOS (LPX1) producing acetate, butyrate and propionate

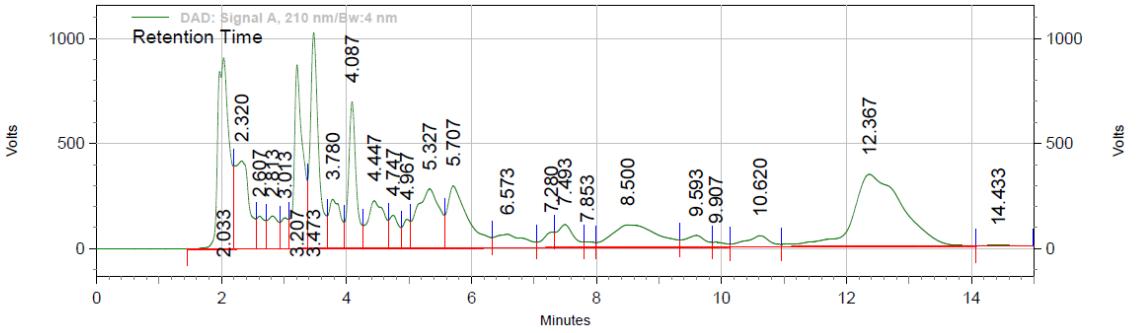


Fig. 5.8.6 (b): HPLC chromatogram of *Lactobacillus plantarum* + XOS (LPX2) producing acetate, butyrate and propionate

5.8. Organoleptic evaluation of *Paneer Butter Masala, Prawn patia, Black rice kheer,* and *Gajar Ka Halwa*

F test revealed no significant difference on the organoleptic scores of XOS added *Paneer Butter Masala* at all levels of addition (5g, 8g and 10g) prepared by substituting sugar with varying levels of XOS (Table 5.8.1).

Table 5.8.1: Organoleptic properties of *Paneer Butter Masala*

Levels of XOS addition		Color & Appearance	Texture	Taste & Mouthfeel	After taste	OA
Std.	Mean SD	8.6 ± 1.4	8.8 ± 1.5	8.2 ± 1.1	8.5 ± 1.1	8.1 ± 1.1
5g	Mean SD	9.0 ± 0.9	9.1 ± 0.9	8.3 ± 0.8	8.8 ± 0.5	8.6 ± 0.7
8g	Mean SD	8.6 ± 1.4	9.2 ± 0.7	8.3 ± 1.5	8.6 ± 0.6	8.6 ± 0.9
10g	Mean SD	9.0 ± 1.0	9.1 ± 0.9	8.5 ± 1.6	8.6 ± 0.9	8.7 ± 1.1
	ANOVA	2.01^{NS}	2.31^{NS}	1.43^{NS}	1.79^{NS}	1.65^{NS}

Significant at *p≤0.05, **p≤0.01, *** p≤0.001, NS- Non significant

Paneer Butter Masala

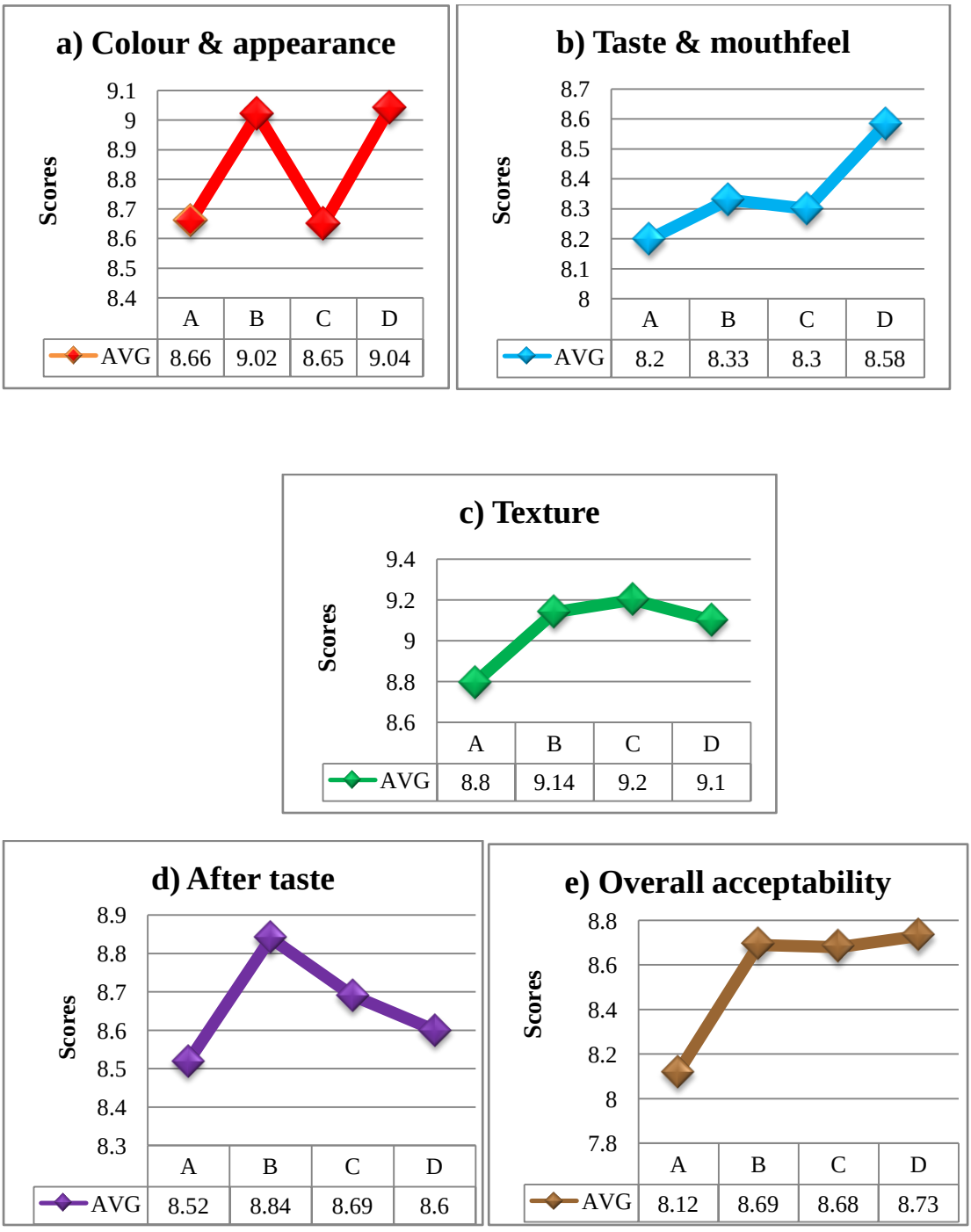


Fig. 5.9.1 (a-e): Scores for organoleptic attributes of *Paneer Butter Masala* with varying levels of added XOS

A= Std. B=5g XOS C=8g XOS D=10g XOS

F test revealed no significant difference in the organoleptic scores of XOS added *Prawn patia* at all levels of addition (5g, 8g and 10g) prepared by substituting sugar with varying levels of XOS (Table 5.8.2).

Table 5.8.2: Organoleptic propertiesof *Prawn patia*

Levels of XOS addition		Color & Appearance	Texture	Taste & Mouthfeel	After taste	OA
Std.	Mean SD	8.8 ± 1.1	9.0 ± 1.2	8.4 ± 1.0	8.4 ± 0.8	8.5 ± 0.8
5g	Mean SD	9.0 ± 0.9	9.2 ± 0.8	8.1 ± 0.8	8.5 ± 0.7	8.6 ± 0.5
8g	Mean SD	8.6 ± 1.3	9.2 ± 0.7	8.0 ± 1.8	8.5 ± 0.7	8.4 ± 1.1
10g	Mean SD	9.1 ± 1.0	9.2 ± 0.8	8.3 ± 1.9	8.5 ± 0.8	8.6 ± 1.3
	ANOVA	2.11^{NS}	2.47^{NS}	1.29^{NS}	1.61^{NS}	1.66^{NS}

Significant at *p≤0.05, **p≤0.01, *** p≤0.001, NS- Non significant

Prawn patia

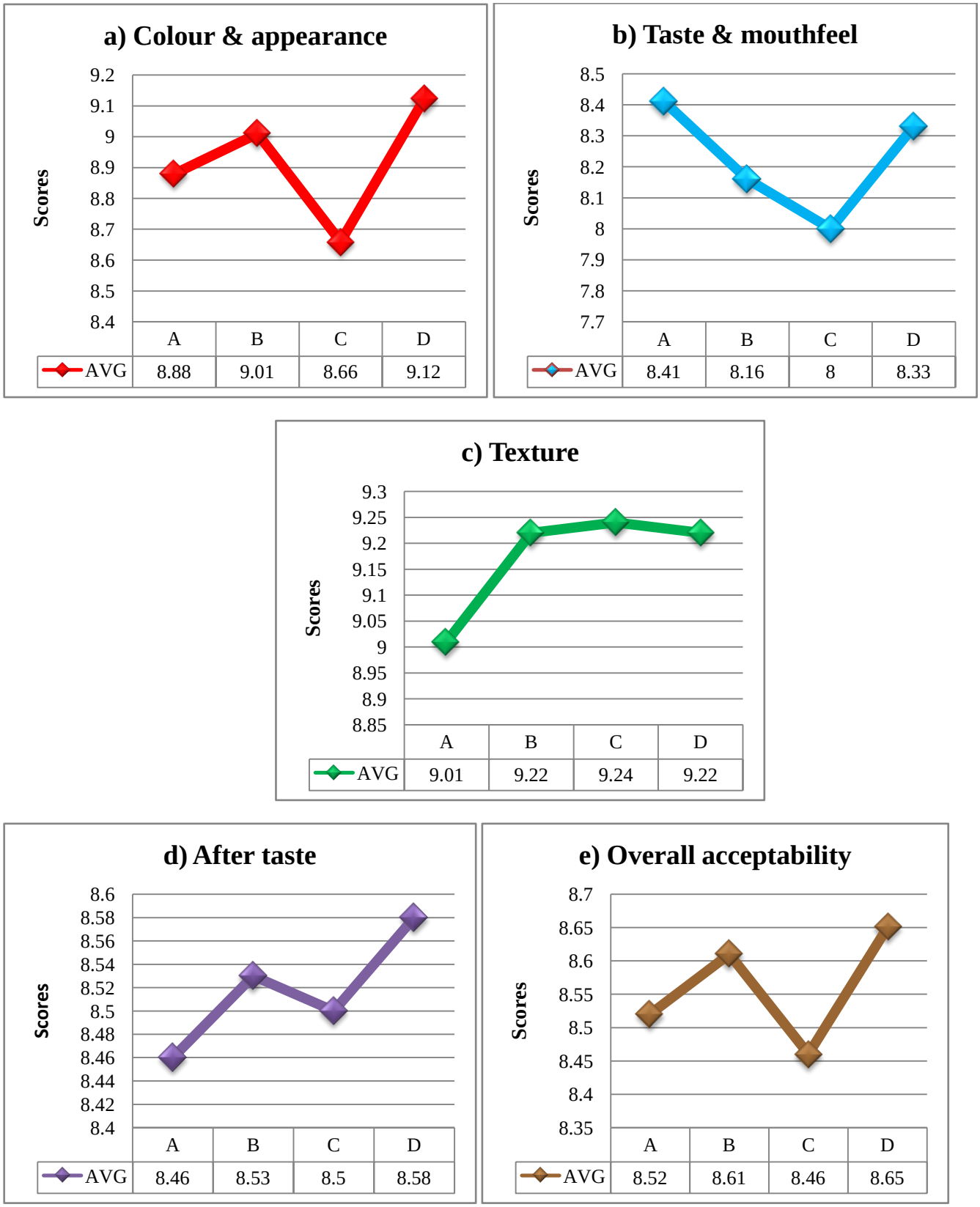


Fig. 5.9.2(a-e): Scores for organoleptic attributes of *Prawn patia* with varying levels of added XOS

A= Std. B=5g XOS C=8g XOS D=10g XOS

F test revealed no significant difference on the organoleptic scores of XOS added *Black rice kheer* at all levels of addition (5g, 8g and 10g) prepared by substituting sugar with varying levels of XOS (Table 5.8.3).

Table 5.8.3: Organoleptic properties of *Black rice kheer*

Levels of XOS addition		Color & Appearance	Texture	Taste & Mouthfeel	After taste	OA
Std.	Mean SD	8.7 ± 0.6	8.3 ± 1.2	8.8 ± 0.9	8.6 ± 0.9	8.6 ± 1.2
5g	Mean SD	7.9 ± 1.3	8.6 ± 1.2	8.2 ± 1.3	8.1 ± 1.2	8.4 ± 1.1
8g	Mean SD	8.0 ± 1.4	8.4 ± 1.1	7.8 ± 1.1	8.0 ± 0.9	7.7 ± 1.0
10g	Mean SD	7.6 ± 1.6	8.5 ± 1.0	7.1 ± 1.0	7.0 ± 1.2	7.4 ± 1.2
	ANOVA	1.14^{NS}	1.59^{NS}	1.07^{NS}	1.01^{NS}	1.08^{NS}

Significant at *p≤0.05, **p≤0.01, *** p≤0.001, NS- Non significant

Black rice kheer

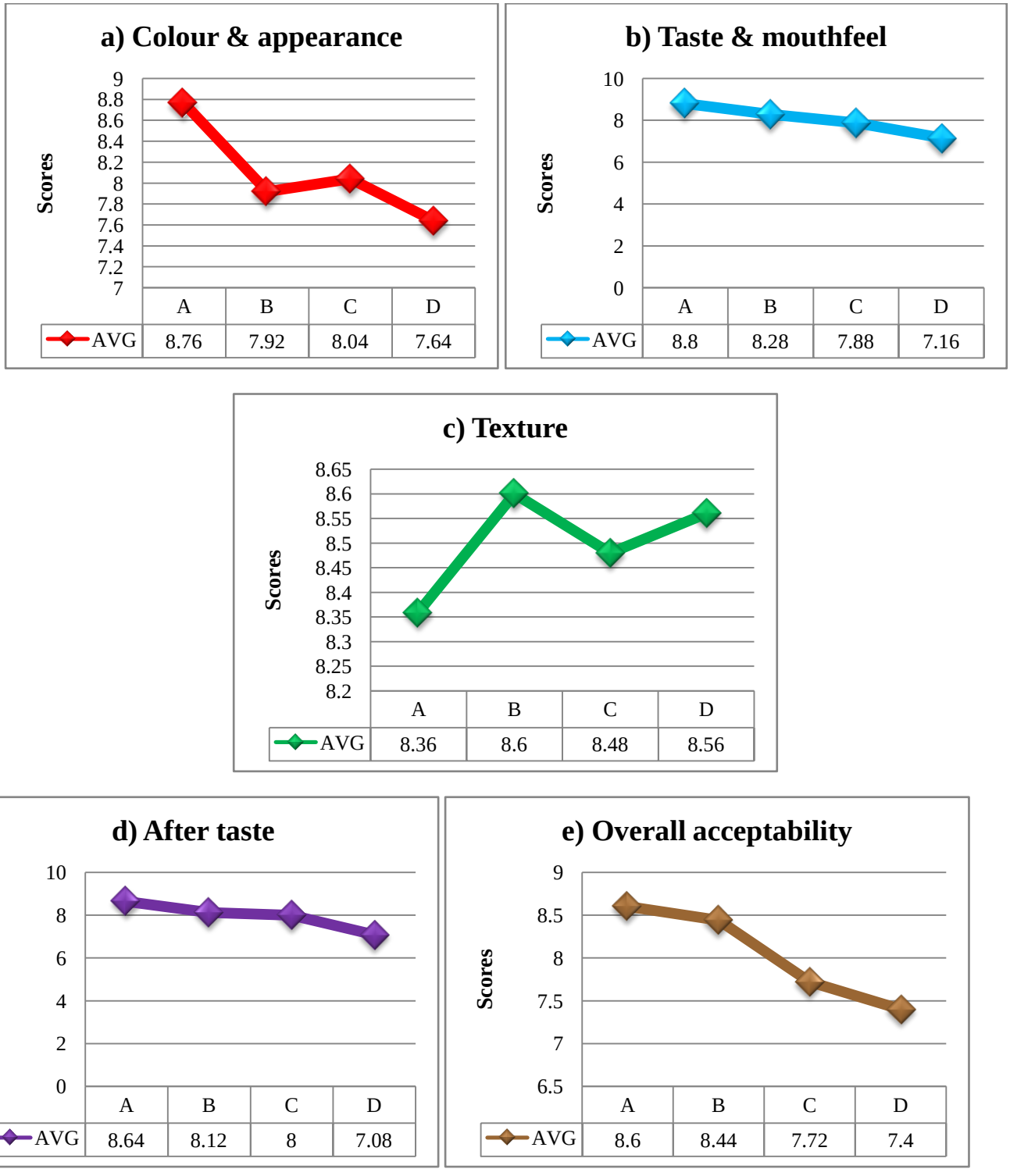


Fig. 5.9.3 (a-e): Scores for organoleptic attributes of *Black rice kheer* with varying levels of added XOS

A= Std. B=5g XOS C=8g XOS D=10g XOS

F test revealed no significant difference on the organoleptic scores of XOS added *Gajar ka halwa* at all levels of addition (5g, 8g and 10g) prepared by substituting sugar with varying levels of XOS (Table 5.8.4).

Table 5.8.4: Organoleptic properties of *Gajar ka halwa*

Levels of XOS addition		Color & Appearance	Texture	Taste & Mouthfeel	After taste	OA
Std.	Mean SD	8.8 ± 0.6	8.1 ± 0.8	8.1 ± 1.2	7.8 ± 1.0	8.1 ± 1.2
5**g**	Mean SD	6.8 ± 1.4	7.5 ± 1.2	8.0 ± 1.3	7.6 ± 1.1	7.5 ± 1.3
8**g**	Mean SD	6.9 ± 1.3	7.4 ± 1.3	7.9 ± 1.0	7.6 ± 0.9	7.2 ± 1.1
10**g**	Mean SD	7.4 ± 0.8	8.1 ± 0.8	7.7 ± 1.0	7.6 ± 1.2	7.5 ± 1.0
	ANOVA	0.62^{NS}	0.88^{NS}	1.02^{NS}	0.79^{NS}	0.74^{NS}

Significant at *p≤0.05, **p≤0.01, *** p≤0.001, NS- Non significant

Gajar ka halwa

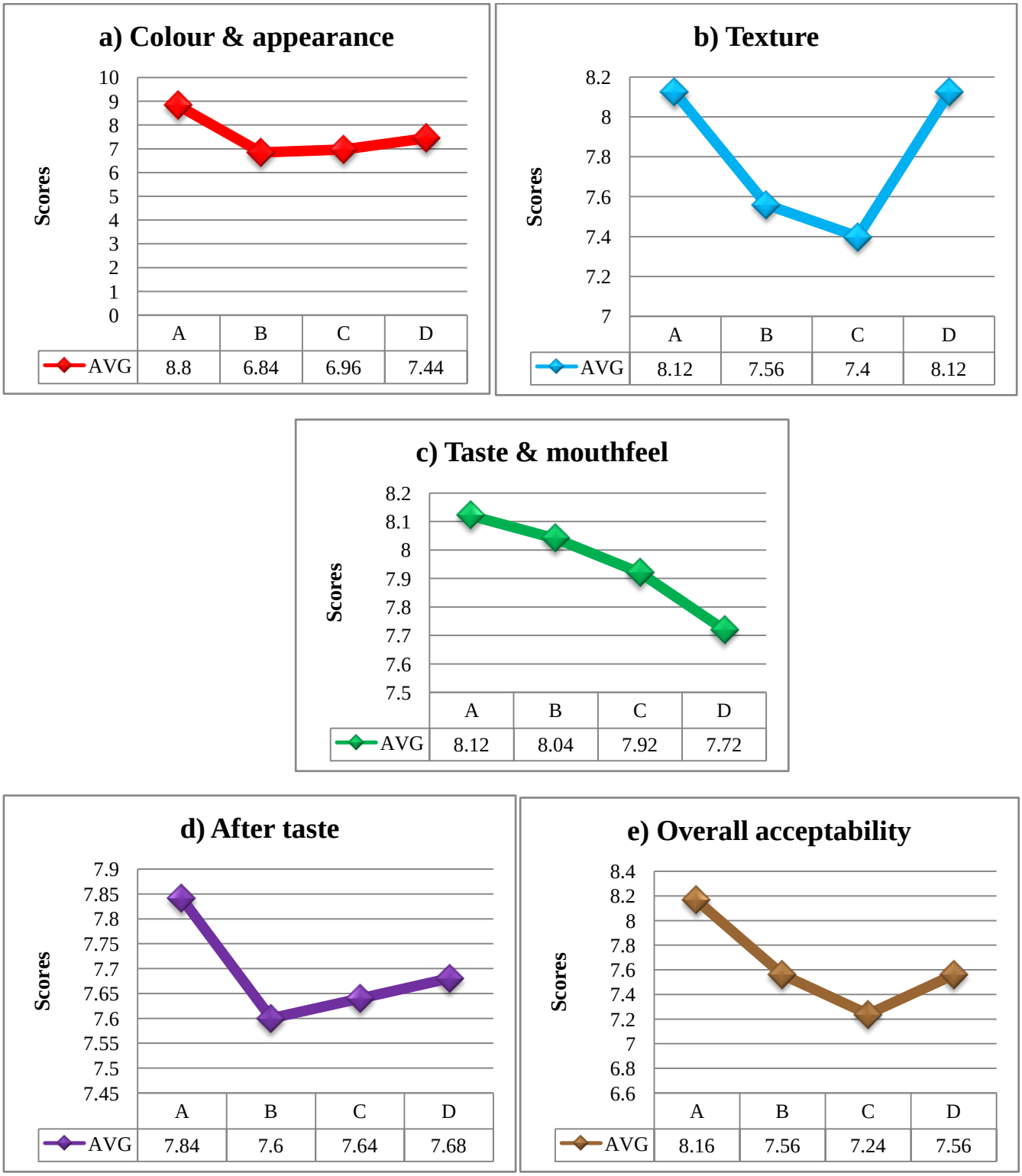


Fig. 5.9.4 (a-e): Scores for organoleptic attributes of *Gajar ka halwa* with varying levels of added XOS

A= Std. B=5g XOS C=8g XOS D=10g XOS

Difference test conducted to determine if the products judged were superior, equal or inferior to the standard product (0% XOS) with varying levels of XOS revealed that in *Paneer Butter Masala*, most of the panelists found its taste to be superior or equal to the standard ($p \leq 0.001$) at all the three levels of addition. The overall acceptability and other sensory attributes of *Paneer Butter Masala* were equal or superior at 8g (Table 5.9.1).

In *Prawn patia,* difference test revealed that the sensory attributes of *Prawn patia* with different levels of addition of XOS were either superior or equal to the standard product ($p \leq 0.001$) (Table 5.9.2).

Table 5.9.3 reveals that most of the panelists found color of the *Black rice kheer* to be superior or equal to the standard ($p \leq 0.01$) at all the three levels of addition. The overall acceptability and other sensory attributes of Black rice kheer were equal or superior at 8g and addition of 10g XOS rendered *Black rice kheer* less sweet.

Most of the panelists found the taste of *Gajar Ka Halwa* to be superior or equal to the standard ($p \leq 0.01$) at all the three levels of addition. The overall acceptability and other sensory attributes of *Gajar Ka Halwa* were equal or superior at 8g and addition of 10g of XOS made it equally acceptable as compared to the standard (Table 5.9.4).

Table 5.9.1: Number of panel members indicating the difference in the organoleptic attributes of *Paneer Butter Masala* in a difference test

Level of substitution	SENSORY ATTRIBUTES															
	Color				Taste				After taste				Overall acceptability			
	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value
Paneer **5%**	7	7	7	0.11^{NS}	0	27	27	2.90***	7	7	0	1.85***	7	13	13	0.006**
8%	61	68	61		68	28	28		68	48	48		61	42	42	
10%	7	0	7		7	20	20		0	20	27		7	20	20	

Level of significance in increasing order- (* p<0.05, ** p<0.01, *** p<0.001)
NS- Non significant

Table 5.9.2: Number of panel members indicating the difference in the organoleptic attributes of *Prawn Patia* in a difference test

Level of substitution	SENSORY ATTRIBUTES															
	Color& appearance				Taste& Mouthfeel				After taste				Overall acceptability			
	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value
5%	0	69	6	0.001***	0	69	6	1.69***	12	63	0	0.0006***	12	63	0	7.04***
8%	12	63	0		36	24	15		12	48	15		24	36	15	
10%	6	63	6		36	21	18		6	54	15		24	33	18	

Level of significance in increasing order- (* p<0.05, ** p<0.01, *** p<0.001)
NS- Non significant

Table 5.9.3: Number of panel members indicating the difference in the organoleptic attributes of *Black rice kheer* in a difference test.

Level of substitution	SENSORY ATTRIBUTES															
	Color& appearance				Taste& mouthfeel				After taste				Overall acceptability			
	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value
5%	0	32	43	0.01**	6	63	6	5.21***	31	32	12	1.01***	31	44	0	4.58***
8%	0	32	43		25	35	15		0	42	33		9	57	0	
10%	6	32	37		25	10	40		22	20	33		6	22	47	

Level of significance in increasing order- (* p<0.05, ** p<0.01, *** p<0.001)
NS- Non significant

Table 5.9.4: Number of panel members indicating the difference in the organoleptic attributes of *Gajar Ka Halwa* in a difference test.

Level of substitution	SENSORY ATTRIBUTES															
	Color				Taste				After taste				Overall acceptability			
	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value	Superior	Equal	Inferior	Chi sq value
Halwa																
5%	21	21	21	0.001***	14	27	20	0.008**	20	14	14	0.46^{NS}	20	20	34	0.0001***
8%	20	27	41		34	14	28		27	27	34		41	28	14	
10%	34	27	13		27	34	27		28	34	27		14	27	27	

Level of significance in increasing order- (* p<0.05, ** p<0.01, *** p<0.001)
NS- Non significant

Result highlights

- XOS obtained from the xylan of 60g corn cob, green banana peel, orange peel and green pea shells were 1.8g (18.75%), 1.01g (18.70%), 1.41g (18.80%)and 0.79g (18.80%) respectively at ($p \leq 0.01$) with an optimal condition of 12h incubation time, pH 5.4 at 40^{o}C.

- No degradation of XOS was observed on exposure of XOS to bile at 0h, 1.5h and 3h with bile concentration 0.5%, 1% and 1.5%.

- XOS recovery was observed to be 100% on its exposure to pH 1.5, 2 and 3 at 0h. At 1.5 h, recovery of XOS was found to be 98.4%, 98.9% and 97.9% at pH 1.5, pH 2 and pH 3 respectively. XOS recovery was 96.2%, 97.3% and 96.3% on its exposure to pH 1.5, pH 2 and pH 3 respectively at 3 h.

- Growth of *Lactobacillus plantarum (LP)* and *Bifidobacterium adolescentis (BA)* were higher at 0.5%, 1% and 2% of XOS addition. For *Escherichia coli (E.coli)* the growth gradually decreased as the concentration of XOS increased from 0.5% to 2%.

- *Bifidobacterium adolescentis* produced 408.6 mMol(↑331%) propionate on its exposure to XOS. *Lactobacillus plantarum* produced 405.62 mMol(↑188%) propionate on its exposure to XOS. When *Escherichia coli* were exposed to XOS production of propionate reduced 339.55 mMol (↓20%).

- *Bifidobacterium adolescentis* produced 343.28 mMol(↑331%) butyrate on its exposure to XOS. *Lactobacillus plantarum* produced 340.72 mMol (↑188%) of butyrate on its exposure to XOS. When *Escherichia coli* were exposed to XOS production of butyrate reduced 285.22 mMol (↓21%).

- *Bifidobacterium adolescentis* produced 1833.27 mMol(↑35%) acetate on its exposure to XOS. *Lactobacillus plantarum* produced 1883.82 mMol of acetate on its exposure to XOS and produced 0 mMol when exposed to glucose. When *Escherichia coli* was exposed to XOS production of acetate reduced to 324.65 mMol (↓48%).

Result highlights

- F test revealed no significant difference on the organoleptic scores of XOS added *Black rice kheer, Gajar Ka Halwa, Paneer Butter Masala* and *Prawn patia* at all levels of addition (5g, 8g and 10g) prepared by substituting sugar with varying levels of XOS.

- In *Paneer Butter Masala*, most of the panellists found its taste to be superior or equal to the standard ($p \leq 0.001$) at all the three levels of addition. The overall acceptability and other sensory attributes of *Paneer Butter Masala* were equal or superior at 8g.

- In *Prawn patia*, difference test revealed that the sensory attributes of *Prawn patia* with different levels of addition of XOS were either superior or equal to the standard product ($p \leq 0.001$).

- Most of the panellists found color of *Black rice kheer* to be superior or equal to the standard ($p \leq 0.01$) at all the three levels of addition. The overall acceptability and other sensory attributes of Black rice kheer were equal or superior at 8g and addition of 10g XOS rendered *Black rice kheer* less sweet.

- Most of the panellists found the taste of *Gajar Ka Halwa* to be superior or equal to the standard ($p \leq 0.01$) at all the three levels of addition. The overall acceptability and other sensory attributes of *Gajar Ka Halwa* were equal or superior at 8g and addition of 10g of XOS made it equally acceptable as compared to the standard.

DISCUSSION

In the present study, different levels of XOS yield were determined from xylan of the four selected agricultural wastes using 4%sodium hydroxide (NaOH). During alkaline extraction, steam application is suggested to enhance the yield of xylan, therefore, in this study the broth was steamed at 100^{o}C for 5h. In the present study, crude xylan yield was 9.60 g (16.0%), 5.40 g(9.0%), 7.50 g (12.5%) and 4.20 g (7.0%), respectively.

The second most available biopolymer of the plant kingdom is Xylan and the major form of hemicelluloses found in agricultural by-products. Xylan has a wide variety of applications in diversified fields which have not been exploited so far (Samanta et al, 2015).

Samanta et al, 2012, attempted to extract the xylan from *S.nervosum* grass with incremental levels (2%, 4%, 8% and 12%) of both sodium hydroxide (NaOH) and potassium hydroxide (KOH).They further investigated the effect of different alkali on the recovery of xylan from particular grass under overnight incubation at room temperature (16h, 25^{o}C) or autoclaving (121^{o}C, 15 lbs, 45min).

They reported that during overnight incubation at room temperature, the incremental levels of either potassium hydroxide or sodium hydroxide resulted in increase in true recovery of xylan from 2.47% to 16.52% and 3.75% to 25.12% of original biomass, respectively. 4% KOH and NaOH yielded 6.28% and8.35% xylan, respectively during overnight incubation.

A similar study by Yang et al, 2007 reported that when corncob, bagasse, wheat bran and peanut shell was exposed to 4% (w/v) NaOH and steamed at 100 °C for 3h, xylan yielded from these samples were12.5%, 15.7% 18.5% and 3.5%, respectively.

In the present study, the extracted xylan was further divided into four equal portions for enzymatic hydrolysis to obtain XOS. Commercial xylanase enzyme (2.0%) procured from Sigma; India was used to hydrolyze xylan. They were exposed to different incubation time such as 4h, 6h, 8h and 12 h with pH 5.5 at 40^{o}C.

A significant rise in the yield of XOS was observed as the incubation time increased from 4h-12h ($p \leq 0.01$) for all the four products. The present study revealed that pure XOS obtained from 100g dry powdered samples of corncob, orange peels, raw green banana, and green pea shells were 3g (18.75%), 2.35 (26.11%), 1.68g (13.46%), and 1.31g (18.80%)

respectively. Although all the four samples yielded high amounts of XOS, orange peels yielded the highest.

There are several processes of production of XOS from xylan. Enzymatic hydrolysis is preferred over others as it neither generates toxic compounds nor requires special equipment (Samanta et al, 2012). Production of XOS from various sources of xylan such as corncob, birchwood, wheat bran and tobacco stalk etc. using commercial xylanases have been reported by many researchers. Fewer attempts were made for production of XOS using indigenously produced xylanases.

A study was conducted in which xylanase was produced using a low cost technique with wheat bran as a substrate and anaerobically treated distillery spent wash as the moistening agent by *A. foetidus* (Chapla et al. 2012).Another study was conducted to produce XOS using orange peels as substrate and the source of enzyme was *Aspergillus niger* (Gupta et al, 2015).

In another study, 3 commercial xylanase preparations (Rapidase Pomaliq from Gist-Brocades, Clarex ML from Generor and Validase from Valley Research) were evaluated as a sole enzyme source for the enzymatic production of pentoses from the hemocellulose fraction of corn husks and corncobs. The results indicated that Rapidase Pomaliq, an enzyme from Aspergillus niger and Trichoderma resei, could serve as the sole enzyme source for the production of pentoses and XOS from corn residues (Achary et al, 2011).

Akpinar et al 2007 found that cotton stalk, which had no economical value, could be converted by enzymatic hydrolysis to a more valuable XOS product. 24 h of hydrolysis yielded 53% XOS at40^{o}C. Another study conducted by Yang et al. 2007 revealed the production of XOS from various xylan obtained from corncob, bagasse, wheat bran and peanut shell by extracellular xylanases from *Thermobifida fusca* NTU22 was 29.5%, 23.7%, 7.6% and10.1%, respectively.

A study conducted by Gupta et al, 2014-2015 reported that the amount of XOS in freeze dried samples of sweet lime peel and orange peel (retentate and permeate) was 190 mg/mL and 333mg/mL, 146 mg/mL and 558 mg/mL, respectively. Therefore, it was concluded that orange peel is the best out of the two substrates for producing XOS.

Another study conducted by Samanta et al, 2015 reported that they found a total concentration of XOS derived from corncob (excluding xylose) varied from 1.19 to 1.69

mg/mL, depending on pH, temperature of reaction, dose of enzyme and duration of hydrolysis.

Whereas, the present study resulted into higher concentration of XOS derived from corncob (79.41mg/mL), orange peels (74.73 mg/mL), green banana peels (73.50 mg/mL)and green pea shells (71.94 mg/mL).

In the present study, no degradation of XOS was observed on exposure of XOS to bile at 0h, 1.5h and 3h with bile concentration 0.5%, 1% and 1.5%. The tests were carried out in duplicates.

A study on pH stability of prebiotic non-digestible wheat bran-derived arabinoxylooligosaccharides (AXOS), xylooligosaccharides (XOS)-and chicory root inulin-derived fructooligosaccharides (FOS) were compared. Decomposition was revealed at alkaline pH (pH 11.0) for all three preparations tested. The short chain oligosaccharides, XOS and FOS were more sensitive to alkaline decomposition than were the longer chain AXOS, the latter being the result of the higher abundance of reducing ends in short chain oligosaccharide preparations (Courtin et al, 2009).

In this study, XOS recovery was observed to be 100% on its exposure to pH 1.5, 2 and 3 at 0h. At 1.5 h, recovery of XOS was found to be 98.4%, 98.9% and 97.9% at pH 1.5, pH 2 and pH 3 respectively. XOS recovery was 96.2%, 97.3% and 96.3% on its exposure to pH 1.5, pH 2 and pH 3 respectively at 3 h. The tests were carried out in duplicates.

At pH 2.0 and 3.0, hydrolysis of oligosaccharide linkages took place, with FOS being the most acid-sensitive component (Courtin et al, 2009). Recoveries were 100%, 91% and 113% for the supplemented muffin, cookie and nutrition bar, respectively at 3.5 pH. For the breakfast cereal, only 47% of the supplemented FOS remained after extrusion at optimal conditions (170 rpm and 140 °C) (Duar, 2011). Whereas, recoveries of Inulin at pH 3.5 were 106%, 103% and 107% and 126% obtained from the supplemented extruded cereal, nutrition bar, sports drink and muffins, respectively (Duar, 2011).

Another study on evaluation of the prebiotic effects of citrus pectin hydrolysate (PEH), it was found that when pH was reduced to 3.2, populations of the tested probiotics did not decrease significantly ($p > 0.05$) for all treatments. The tested probiotics showed

significantly higher acid tolerance and survival populations in the media supplemented with PEH than glucose. This indicated that PEH should contain some oligosaccharides which assisted the probiotics in acid tolerance and survival ability, while glucose did not (Yen et al, 2017).

Cummings et al, 2001 reviewed on the digestibility of Inulin and Oligofructose and found an average recovery of 88% in human upper intestine. There is little available information in the literature on bile resistance, acid tolerance properties of XOS *in vitro.*

In this study, the growth of *Lactobacillus plantarum (LP)* and *Bifidobacterium adolescentis (BA)* were higher at 0.5%, 1% and 2% of XOS addition. For *Escherichia coli (E.coli)* the growth gradually decreased as the concentration of XOS increased from 0.5% to 2%. Since 0.5%, 1% and 2% levels of XOS concentration gave better or almost equivalent growth of *Lactobacillus plantarum (LP), Bifidobacterium adolescentis (BA)* and reduced the growth of *Escherichia coli (E. coli).* Therefore, 0.5%, 1% and 2% levels of XOS concentration samples were chosen for production of short chain fatty acids (SCFA) and its analysis.

Mean growth of *Lactobacillus plantarum* was more with 0.5% and 1% XOS concentration at $p \leq 0.01$, growth of *Bifidobacterium adolescentis* was seen to be same with 0.5% and 2% XOS concentration at $p \leq 0.01$ and growth of *Escherichia coli* was least with 1% XOS.

A study on functional properties of commercial prebiotics showed the increase in cell density of *L. paracasei 1195* grown on Raftilose P95, Inulin-S, and Raftiline HP were significantly higher ($p \leq 0.05$) than for glucose. *B. bifidum NCI* had a significantly higher ($p \leq 0.05$) increase in cell density when grown on NutraFlora P-95 and Raftilose P95 than on glucose. Also, the increase in cell densities of *L. plantarum 4008* and *L. acidophilus 33200* were significantly larger ($p \leq 0.05$) for purified GOS than for glucose (Huebner et al, 2007).

An in vitro study investigated the potential prebiotic effect of natural (NS) and blanched (BS) almond skins, the latter being a by-product of the almond-processing industry. Their study concluded that dietary fibre from almond skins altered the composition of gut bacteria and almond skins resulting from industrial blanching could be used as potential prebiotics (Mandalari et al, 2009).

A study on the prebiotic activity of XOS obtained from corncob and reagent grade xylan were tested in *L. brevis, L. plantarum, L. acidophilus, L. rhamnosus* cultures, and in a co-culture with *Escherichia coli* as a challenge microorganism to prove the bacteriostatic

activity of lactobacilli strains. Xylooligosaccharides stimulated *L. brevis* and *L. plantarum* growth: these microorganisms grew faster than the other lactobacilli strains. *L. acidophilus* grew better in the presence of XOS and maintained the absorbance of the culture. In the co-culture in presence of both XOS the challenge microorganism did not grow; lactobacilli colonies appeared in MRS agar. No colonies of *E. coli* grew in EMB plaques (Pedraza et al, 2014).

Lactobacilli and Bifidobacteria ferment carbohydrates through a pathway mediated by the glycolytic enzymes in which the main end products are SCFA (Grootaert et al, 2007). Butyrate, Propionate and Acetate are the major SCFA produced during fermentation of carbohydrates in the large bowel (Maniserri et al, 2009).

A study on bioactive xylooligosaccharides from wheat bran soluble polysaccharides reported that Acetate was the chief SCFA liberated due to in vitro fermentation of xylooligosaccharides (Maniserri et al, 2009).

An in vitro study revealed the comparison of XOS fermentation from corn cob and commercial XOS by *Bifidobacterium adolescentis, B. longum, Lactobacillus brevis* and *L. fermentum. B. adolescentis* and *L. brevis* grew highest on XOS; *B. longum* and *L. fermentum* grew least on XOS (Moura et al, 2007).

In an in vitro study, corn cob generated XOS was assessed by enumerating the colony forming units for four proven probiotic strains revealed that prebiotic action of XOS was higher with probiotics *Enterococcus faecium* followed by *Enterococcus fecalis, Lactobacillus maltromicus, Lactobacillus viridiscens* implicating variable growth stimulatory effect of XOS (Samanta et al, 2012).

Corn cob XOS tested the prebiotic activity of *Lactobacillus plantarum* and found that cells were denser and their growth rates were higher when cultured on XOS. Acetate was found to be the major short-chain fatty acid produced as the end-product of fermentation (Xiuhua Yu et al, 2015).

Another study on prebiotic effects of Xylooligosaccharides on the improvement of microbiota balance in human subjects reported that the abundance of pathogenic bacteria, *Clostridium perfringens*, was significantly lower in the fecal samples of the XOS group than

in those of the control group. This was explained by the XOS suppressing the growth of *Clostridium perfringens*; the mechanisms underlying this effect were likely due to the production of short-chain fatty acids (SCFAs) via the fermentation of XOS in the colon. A decrease in intestinal pH has been reported as a consequence of the increased SCFA production which subsequently inhibits the overgrowth of pathogenic bacteria (Lin et al, 2016).

A comparative study of synbiotic and prebiotic supplementation on gut health, SCFA, hs-CRP and lipid profile of type 2 diabetic subjects with pre hypertension concluded that daily intake of 1 g synbiotic product and 10 ml FOS improved gut health, hs-CRP, lipid profile and short chain fatty acids (SCFA) of the subjects which may be due to increased production of SCFA (Sheth et al, 2016).

Another study on consumption of XOS in combination with inulin did not decrease the concentrations of acetate and *p*-cresol, but increased the faecal concentrations of total SCFA and propionate (Lecerf et al, 2012).

In an in vivo study, 16 mice diet were supplemented (1%) with nine different oligosaccharides for a 6- month study period. XOS increased lactobacilli and *Bifidobacteria* counts, reduced sulphite reducing clostridia (Santos et al, 2006).

Another study on XOS in diabetic Wistar rats (150–160g); control group fed with the basal diet for 6 weeks improved body weight, reduced hyperglycaemia, cholesterol, severe glucosuria, proteinuria, diabetic nephropathy, blood creatinine and urea concentrations (Gobinath et al, 2010).

A mice study reported that six-week-old obese mice fed with oligofructose (0.3g/day) feeding (n = 10/group) for 5 weeks decreased firmicutes, improved glucose tolerance, reduced fat accumulation (Everard et al, 2011).

In this study, *Bifidobacterium adolescentis* produced 408.6 mMol(↑331%) propionate on its exposure to XOS. *Lactobacillus plantarum* produced 405.62 mMol(↑188%) propionate on its exposure to XOS. When *Escherichia coli* were exposed to XOS production of propionate reduced 339.55 mMol (↓20%).

It also reveals that *Bifidobacterium adolescentis* produced 343.28 mMol (↑331%) of butyrate on its exposure to XOS. *Lactobacillus plantarum* produced 340.72 mMol (↑188%) of butyrate on its exposure to XOS. When *Escherichia coli* were exposed to XOS production of butyrate reduced 285.22 mMol (↓21%).

Bifidobacterium adolescentis produced 1833.27 mMol (↑35%) of acetate on its exposure to XOS. *Lactobacillus plantarum* produced 1883.82 mMol of acetate on its exposure to XOS and produced 0 mMol when exposed to glucose. When *Escherichia coli* were exposed to XOS production of acetate reduced 324.65 mMol (↓ 48%).

Hence, it can be observed that Acetate was produced the most followed by Propionate and Butyrate. *Bifidobacterium adolescentis* produced acetate (1833mMol), butyrate (343.28mMol) and propionate (408.67mMol).
Lactobacillus plantarum produced acetate (1883.82 mMol), butyrate (340.72mMol) and propionate (405.62mMol).
Escherichia coli produced acetate (324 mMol), butyrate (339.55 mMol) and propionate (285.22 mMol).
Bifidobacterium adolescentis produced (331%) more of Butyrate and Propionate respectively on its exposure to XOS ($p \leq 0.01$), whereas, *Lactobacillus plantarum* produced more acetate as compared to *Bifidobacterium adolescentis* ($p \leq 0.001$). Production of all the three SCFA reduced (20%-48%) in case of *Escherichia coli* on its exposure to XOS ($p \leq 0.001$).

In this study, F test revealed no significant difference on the organoleptic scores of XOS added *Black rice kheer, Gajar Ka Halwa, Paneer Butter Masala* and *Prawn patia* at all levels of addition (5g, 8g and 10g) prepared by substituting sugar with varying levels of XOS. Hence, XOS addition to these products was well accepted by the panelists up to 10g level of addition.

A study conducted on development and sensory analysis of a buttermilk based fermented beverage using barley and fructooligosaccharide as functional ingredients reported high scores for overall acceptability and the sweet taste of FOS did not negatively affect the taste, aftertaste and mouthfeel of the product (Sheth et al, 2016). Another study conducted on FOS added beverages and soup namely, butter milk, lemon juice, milk and tomato soup at 2.5%, 4%, 5%, 6%, 7.5% showed positive results on the overall acceptability of the products (Garg et al, 2011). Similar results were reported by Parnami *et al*, where cookies and bread were fortified with prebiotic inulin (Parnami et al, 2010).

A study on Xylooligosaccharide enriched yoghurt reported that addition of XOS up to 3.5% did not influence taste and overall acceptability but higher levels of addition resulted in lower after taste scores (Mumtaz et al, 2008).

Liquid milk with 0.5–4.0 g/100 g XOS content was stored for 4 weeks and 83% of the initial XOS content was measured at the end of the storage period. The XOS content (2 g/100 g XOS) in yoghurt and powdered milk was reported to be maintained (more than 95%) after 16 days and 5 months, respectively (GRAS, 2013).

In different exemplary foods prepared in a pilot plant, no significant changes of the total contents of XOS 95P, XOS 70P and XOS 70L in the following foods under the indicated storage conditions were observed: Yoghurt (pH 4.6) with XOS 95P (0.34 g/100 g), XOS 70P (0.36 g/100 g), XOS 70L (0.37 g/100 g), respectively; stored for 2 weeks at 4°C; Fruit jelly (pH 3.0) with XOS 95P (2.76 g/100 g), XOS 70P (2.73 g/100 g), XOS 70L (2.69 g/100 g), respectively; stored for 4 weeks at 20°C; Soy drink with XOS 95P (0.33 g/100 g), XOS 70P (0.35 g/100 g), XOS 70L (0.35 g/100 g), respectively; stored for 3 weeks at 4°C; Biscuits with XOS 95P (1.85 g/100 g), XOS 70P (1.85 g/100 g), XOS 70L (1.85 g/100 g), respectively; stored for 2 weeks at room temperature (EFSA Journal, 2018).

A review study mentioned that XOS could be used in soy milk, soft drinks, tea, cocoa drinks, nutritive preparations, dairy products like milk, milk powder, yogurt, candies, jellies, jam and honey products to formulate health foods for elder people and children (Jain et al, 2015).

In the present study, difference test conducted to determine if the products judged were superior, equal or inferior to the standard product (0% XOS) with varying levels of XOS revealed that in *Paneer Butter Masala*, most of the panelists found its taste to be superior or equal to the standard ($p \leq 0.001$) at all the three levels of addition. The overall acceptability and other sensory attributes of *Paneer Butter Masala* were equal or superior at 8g.

In *Prawn patia*, difference test revealed that the sensory attributes of *Prawn patia* with different levels of addition of XOS were either superior or equal to the standard product ($p \leq 0.001$).

Most of the panelists found color of the *Black rice kheer* to be superior or equal to the standard ($p \leq 0.01$) at all the three levels of addition. The overall acceptability and other

sensory attributes of Black rice kheer were equal or superior at 8g and addition of 10g XOS rendered *Black rice kheer* less sweet.

However, most of the panelists found the taste of *Gajar Ka Halwa* to be superior or equal to the standard ($p \leq 0.01$) at all the three levels of addition. The overall acceptability and other sensory attributes of *Gajar Ka Halwa* were equal or superior at 8g and addition of 10g of XOS made it equally acceptable as compared to the standard.

The following null hypotheses formulated at the beginning of the study are rejected:

- The four agricultural wastes will not yield different amount of XOS.
- XOS will not show prebiotic properties in the in vitro trial.
- XOS added Indian traditional foods will not be accepted by the panellists for most of the organoleptic attributes.

The following alternate hypotheses have been accepted:

- The four agricultural wastes will yield different amount of XOS.
- XOS will show prebiotic properties in the in vitro trial.
- XOS added Indian traditional foods will be accepted by the panellists for most of the organoleptic attributes.