Development of Enriched Yoghurt With Cabbage *(Brassica oleraceae L.var capitato)* Powder and Its Antimicrobial Property

[1]Ashwini B.Bangar, [2]M.I.Talib, 3Vishal R.Parate
Assistant Professor UICT, KBC-North Maharashtra University Jalgaon.
[1]ashwinibangar38@gmail.com, [2].drmtalib@gmail.com, [3]vishal_parate@yahoo.com

Abstract

Study of plants phytochemicals has recently been a great interest in the processed foods. Some plant phytochemicals or biomolecules also exhibit antimicrobial properties and may help to avert food microbial intoxication. In present work shredded cabbage leaves were dried and converted into cabbage powder, and extract was prepared in three pure solvents viz: ethanol, methanol acetone and also in their aqueous solution of 20% ,40% ,60% and 80% concentrations. All extracts were tested for their sensitivity against E.coli (NCIM No.2065), staphylococcus aureus (2127), salmonella enteric (NCIM No.5284). The disc diffusion method was adopted for testing antimicrobial activity of extacts against E.coli, staphylococcus aureus, salmonella enteric. All prepared cabbage powder extracts showed sensitivity against three tested bacterial cultures, pure acetone extract showed highest efficiency against tested bacterial cultures than methanol and ethanol extract and that sensitivity decreased with dilution of the solvents used for extraction. Four yoghurt samples were prepared by incorporating 2.2 g%, 4.5g% , 9g% and 18g% cabbage powder and were marked as FY2.2,FY4.5,FY9and FY18 respectively and were compared with plain control yogurt(Y0).It was reported that the FY9 yoghurt was sensory acceptable among all yoghurt samples and it was enriched with gallic acid(2.5mg%) ,quercetin(0.5mg%) and rutin (15 mg%) with respect to plain yoghurt. Sensory acceptable yoghurt (FY9) exhibited zone of inhibition of 12.3mm, 15.5m and 13.2 mm against E.coli, staphylococcus aureus and salmonella enteric respectively. All the yoghurt samples were reported to be palatable for fifteen days at refrigerated temperature and their total plate count (3.26×10^3), yeast and mold counts (2.54×10^3) were within permissible limit with no coliform count detected. It may be concluded that the 9g% cabbage powder fortified yogurt may help to alleviate salmonellosis, staph poisoning and gastro enteritis; and may provide substantial source of health benefitting phytochemicals with probiotics alike functional yoghurt.

Keywords: **Phytochemicals, Yoghurt, Antimicrobial activity, Shelf life.**

1. INTRODUCTION

Antimicrobial properties of various plant extracts have open a new avenue in research with their possible use as natural additives to replace synthetic antimicrobial agent (Prasad M.P.et al., 2014). Antimicrobial active plant components are capable of destroying or inhibiting the growth of microorganism(S.Sewani et al., 2016).Vegetable may be deemed as potent medicine and man is able to obtain from them a wondrous assortment of industrial chemical(Suganya.D et al., 2016).The pathogens impose resistant to antibiotics due to indiscriminate use of modern antibiotic (Salvet,A et al., 2001).The active biomolecule of herbs and spices act against microorganism and secondary metabolites(i.e. alkaloid,flavonoid and glycoside) (Yadav.R et al., 2016).

In present study an attempt is made to investigate and exploit antimicrobial potential of cabbage powder for development of functional yogart.

2. MATERIALS AND METHOD

The cabbage was procured from local market of Jalgaon and was analysed for its composition as per standard laboratory methods of S.Ranganna, 1986, while crude fibre was estimated on fibre analyser (Pelican KES 04LVA). Culture tubes of *Staphylococcus aureus (NCIM*

No.2065). Salmonella enteric ((NCIM No.5284)) and *Escherichia coli (NCIM No.2065)* were procured from National Chemical Laboratory (NCL) Pune. DVS yoghurt starter (YC-X11), and DVS probiotic starter *(B.lactic and S.thermophillus)* were obtained from Chr- Hansen Co. Fat content of yogart was determined by Garber method (Kurt A.). The polyphenolics were determined by HPLC method (Jaiswal et al., 2011).

Preparation of Methanol, Ethanol, Acetone and water extract :

Cabbage Extract in methanol, ethanol and acetone was prepared by mixing 30grams of dry cabbage powder in 300ml of solvent, and 48 hours of extraction time was used at ambient temperature. Aqueous cabbage extract was also prepared in 1:5 ratio of cabbage powder in boiling for 2hours. Filtered extract was used for assessing antimicrobial activity (B.Pandey, S.Kumari et al., 2013).

Preparation of culture broth:

Nutrient broth was used for the preparation of culture media. One loop full of culture was mixed in 25ml of sterile nutrient broth and incubated in shaking incubator for 24 hours.

Assessment of antimicrobial activity:

Disc diffusion method was used for the evaluation of antimicrobial activity. Sterile paper disc of 6mm diameter soaked in cabbage extract, and it was aseptically placed on lawn of inoculum on nurient agar plate. The plates were then incubated at 37^{o}C for 24 to 48 hrs.After 24 hours the plates were examined for zone of inhibition around paper disc.

Preparation of cabbage powder:

Cabbage leaves were washed with clean water and cut into shredds of of about 20mm×20mm dimensions. Shredded leaves were then dried in hot air oven maintained at 60^{o}C to 8 to 9 g % moisture content. The dried cabbage leaves were then grinded to powder form and sieved through 60 mesh size . Cabbage powder was packed in airtight polyethylene bags.

Preparation of yoghurt:

Fresh buffalow milk was boiled and slightly concentrated to 80% original volume. At the time of boiling 10% sugar was added and stirred continuously by using stirrer to prevent the formation of creamy layer. Milk was cooled to 40-45□C and five yogart samples and one plain yogart sample was prepared in small cups. Four yogart samples were prepared by adding 2.2, 4.5, 9 and 18 %(w/v) cabbage powder and inoculated with Starter culture (1:1 v/v %) at 41□C temperature and, finally incubated at 37□C for 8-10 hours to get yoghurt.

Shelf life study of Control yoghurt and cabbage powder enriched yoghurt:

The shelf life study of five samples of yogart was carried out with five days interval upto 15 days at refregerated temperature. During shelf life study all yoghurt samples were tested for total pate count (TPC), Coliform count and yeast and mold count by using standard microbiological techniques. Samples were sensory evaluated on nine- point hedonic scale.

3. RESULTS AND DISCUSSION

Table 1. composition of cabbage powder

Parameters	Results
Moisture	8.17±0.21
Protein	6.36±0.06
Ash	9.68±0.29
Fat	3.26±0.08
Carbohydrate	72.51±0.23
Total Dietary Fibre (%)	59.23±0.04
Total phenols(mg/100ml)	2.91
Flavonoid(mg/100ml)	0.587

Table 2.Functional Properties of Cabbage powder

Parameters	Results
Water Holding capacity(gm)	2.82±0.48
Water Solubility Index (%)	34.95±1.65
Swelling Capacity	2.67±0.01
Viscosity(mPa.a)	0.8872

Cabbage powder is rich in dietary fibre (59.23%), and phytochemicals (2.91 mg gallic acid and 0.587 mg flavonoid..Results are comparable with reported by Prokopov.T et al., 2015.

The water solubility index of cabbage powder was 34.95% (AACC method) and Swelling capacity of cabbage powder was reported to be 2.67,which was comparable with Zang.Z.et al., 2011.While water holding capacity of cabbage powder was found to be 2.82gm/100gm.

The qualitative technique was used for detection of phytochemicals. Alkaloid, Flavonoid, glycoside and total phenols were reported in cabbage powder while Saponin and terpenoid were absent. Results are similar to reported by Suganya et al., 2016.

Table 3. Antimicrobial Activity of Cabbage extract

Concentration	Extract					
	Aqueous			Methanol		
	E.coli	S.aureus	S.enterica	E.coli	S.aureus	S.enterica
100%	12.1±0.55	10.4±0.91	10.86±0.35	17.1±1.04	14.7±0.75	14.1±0.36
80%	10.7±0.66	9.5±0.29	9.8±0.2	15.1±1.25	11.3±1.04	13±0.55
60%	9.5±0.30	8.4±0.65	9.2±0.40	12.6±1.70	10.6±1.05	12.5±0.51
40%	7.36±0.41	7.2±0.26	8.1±0.51	11.63±1.15	9.6±1.01	10.7±0.87
20%	4.46±0.64	5.46±0.66	6.5±0.36	7.5±0.5	9.4±0.85	7.6±0.79
Concentration	Extract					
	Ethanol					

				Acetone		
	E.coli	**S.aureus**	**S.enterica**	**E.coli**	**S.aureus**	**S.enterica**
100%	13±2.64	14.9±0.45	14.8±0.76	24.1±2.02	24.6±3.05	19.4±0.90
80%	12±0.5	13.4±0.65	13.7±0.25	23.7±0.40	24.2±0.75	17.7±0.75
60%	11.3±0.70	13.1±0.36	11.5±0.75	18.2±0.53	19.1±1.04	14.9±0.70
40%	10.7±0.80	12.2±0.87	10.4±0.92	14.8±1.25	14.2±1.05	13.4±0.49
20%	8.4±0.95	7.8±0.28	6.9±0.40	10.6±0.76	12.5±0.50	10.9±0.36

Acetone extract showed highest antimicrobial activity than aqueous, ethanol and methanol extract (Prasad.M.P et al., 2014). Ethanol showed the least antimicrobial activity. All extracts showed significant antimicrobial activity against culture strains used.
It may be attributed to presence of quinines, falvonoids, polyphenols and tannin, alkaloid and glucosinolates of cabbage present in extract (Prashant Tiwari et al., 2011).

Table 4. Physico-chemical composition of Control yoghurt and Cabbage powder enriched yoghurt

Parameter	Control	FY2.2	FY4.5	FY9	FY18
Acidity	0.68±0.08	0.66±0.05	0.63±0.04	0.61±0.04	0.64±0.02
pH	4.26±0.12	4.63±0.26	4.66±0.27	4.74±0.11	4.62±0.10
Moisture (%)	86.02±1.63	87.84±0.83	86.63±0.82	85.46±0.82	86.16±1.81
Fat (%)	4.19±0.07	3.4±0.81	3.63±0.44	3.93±0.25	4.00±0.21
protein	4.26±0.06	4.22±0.10	4.26±0.05	4.46±0.03	4.29±0.23
Ash (%)	0.70±0.002	0.73±0.01	0.74±0.005	0.766±0.001	0.759±0.006
Carbohydrate (%)	4.93±1.66	3.81±1.53	4.73±0.35	5.36±1.05	4.79±1.72
Total solid	22.89±3.52	23.39±1.80	24.94±0.45	25.92±0.18	25.30±0.64
Reducing sugar	5.59±0.25	5.86±0.39	5.90±0.22	6.16±0.19	6.17±0.18
Non-reducing sugar	6.54±0.05	6.47±0.06	6.66±0.21	6.46±0.24	6.53±0.35
Total sugar	12.13±0.21	12.33±0.40	12.57±0.01	12.63±0.16	12.70±0.21
Gallic acid	-	-	-	2.58mg	-
Quercetine	-	-	-	0.588mg	-

Rutin	-	-	-	15.11mg	-

FY 2.2=2.2% fortified cabbage powder Yoghurt , **FY4.5**=4.5% fortified cabbage powder Yoghurt, **FY9**=9% fortified cabbage powder Yoghurt **, FY18**=18% fortified cabbage powder Yoghurt.

The addition of cabbage powder decrease the acidity of yoghurt as compared to control yoghurt. The reported acidity of fortified yoghurt samples were reported to be in range from 0.67 to 0.61 % ; with minimum acidity (0.61%) for FY9 yogart ; and maximum (0.68%) for plain yoghurt (Dbashis et al., 2016). There was no significant difference reported, in chemical composition of plain yoghurt and fortified yoghurt samples and, that 9% cabbage powder fortified yoghurt (FY9) was found to be sensory acceptable.

Table 5 .Inhibition Zone of Cabbage powder fortified yoghurt against pathogenic bacteria

	Zone of Inhibition(mm)		
	E.coli	**S.aureus**	**Salmonella enteric**
Control	8.1±0.37	9.1±0.5	10±0.2
FY2.2	10.2±0.40	13.2±0.4	11.6±0.3
FY4.5	11.3±0.40	14.1±0.25	12.1±0.45
FY9	12.3±0.36	15.3±0.32	13.2±0.32
FY18	12.8±0.35	14.5±0.30	12.6±0.70

L.acidophilus and *S.thermophillus* of yoghurt ,symbiotaically exhibited antimicrobial activity (Chuayana et al., 2003). All yoghurt samples are reported to be sensitive aginst *Escherichia coli, Staphylococcus aureus and Salmonella enterica.* The cumulative antimicrobial properties of yoghurt may be attributed to symbiotic effect of *L.acidophilus*, *S.thermophillus* and cabbage extract. Among all samples of yoghurt, FY9 yoghurt exhibited comparative more sensitive against all tested bacterial cultures.

Table 6. Microbial analysis of control yoghurt and cabbage powder fortified yoghurt

Treatment	**Storage period**					
	0 day			**5day**		
	TPC	**Coliform**	**Y and M**	**TPC**	**Coliform**	**Y and M**
Control	1.60×10^4	Nil	2.00×10^2	1.67×10^4	Nil	2.10×10^2
FY2.2	1.64×10^3	Nil	1.77×10^2	1.68×10^3	Nil	1.79×10^2
FY4.5	1.82×10^3	Nil	2.27×10^2	1.85×10^3	Nil	2.32×10^2
FY9	3.09×10^2	Nil	2.43×10^2	3.14×10^2	Nil	2.47×10^2
FY18	3.69×10^2	Nil	2.00×10^2	3.72×10^2	Nil	2.10×10^2

Treatment	**Storage period**					
	10 day			**15day**		
	TPC	**Coliform**	**Y and M**	**TPC**	**Coliform**	**Y and M**
Control	1.69×10^4	Nil	2.17×10^3	1.73×10^4	Nil	2.20×10^3

FY2.2	1.71×10^3	Nil	1.81×10^2	1.73×10^3	Nil	1.86×10^2
FY4.5	1.88×10^3	Nil	2.36×10^2	2.92×10^3	Nil	2.34×10^2
FY9	3.21×10^2	Nil	2.52×10^3	3.26×10^3	Nil	2.54×10^3
FY18	3.76×10^2	Nil	2.18×10^2	3.79×10^3	Nil	2.98×10^3

TPC=total plate count, **Y and M**=yeast and mold.

The decrease in the concentration of total bacterial count may be due to higher phytochemical content in fortified yoghurt (Amal et al., 2016).The cabbage is source of various phytochemicals (Campbell et al., 2012). The refrigerated shelf life of plain yoghurt was reported to be upto 5 to 6 day, while for cabbage powder fortified yoghurt it was upto 10 days. Alkaloid of extract may be dominant in increasing the shelf life of yogurt (Amal et al., 2016). **It was also** noticed that all yoghurt samples were free from Coliform bacteria (Amal, A.Matter et al., 2016). Higher solid and fibre content of cabbage powder may be contributing to body and textural properties of yogurt (Debashis et al., 2015).

4. CONCLUSION

Sensory acceptable ,cabbage fortified Yoghurt can be prepared by incorporating maximum 9 g% of cabbage powder in yoghurt formulation. Cabbage powder yoghurt so prepared showed sensitivity against pathogenic bacterial cultures:, *E.coli (NCIM No.2065), staphylococcus aureus (2127), salmonella enteric (NCIM No.5284).*Moreover the fortified yoghurt was reported to be enriched with 2.58mg % gallic acid, 0.588 mg% querceitin and 15.11mg % rutin. Cabbage powder incorporated yoghurt can be palatable upto 10 days held at refregerated temperature.

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