

Evaluation of nutraceutical properties of Jamun squash Probioticated using kefir seeds

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ABSTRACT

An attempt was made to formulate nutritious probiotic functional drink through fermentation of Jamun squash using kefir seeds. Initially, physio-chemical parameters of Jamun fruits were evaluated to enhance the processing of squash. Kefir seeds were produced by inoculating kefir grains in pasteurised cow milk and it was subjected to biochemical characterization and presence of probiotic bacteria was confirmed. Probiotic Jamun squash (PJS) was prepared by mixing pasteurized fruit juice along with sugar syrup and inoculated with kefir seeds. Sensory evaluation of PJS gave 90% acceptability by panel members after 72hrs of fermentation duration at 10°C. Nutritional composition such as total carbohydrate, protein, cholesterol and vitamin C content were estimated for PJS, Jamun Squash (JS) and Kefir seeds. The results of total antioxidant content (TAC), total phenolic content (TPC) and total flavonoid content (TFC) were found to be significant for PJS when compared to JS. Similarly, PJS was tested for antimicrobial activity against four different species and maximum zone of inhibition (20mm) was obtained against *Salmonella typhi*. The antidiabetic activity was performed through α -amylase inhibition assay and percentage of inhibition for JS, Kefir seeds and PJS was to be 40, 38 and 47% respectively. Similarly shelf life study of PSJ gave favourable results. Thus the probiotic Jamun squash can be used as a novel product with various beneficial effects and this study could promote commercialization of PSJ as functional drink and pharmaceutical supplement.

Key words: Probiotic jamun squash, kefir seeds, antioxidant, sensory evaluation, functional foods

INTRODUCTION

Demands for production of healthy functional foods are increasing vastly. Apart from providing basic nutrition, functional foods promote health and reduce the risk of diseases. Probiotic foods have conquered major place in functional food market (Baboota *et al.*, 2013) and it acts as a live microbial supplement which helps in renovating microbial balance in the case of intestinal dysfunctions. Probiotic foods improve immunological, digestive and respiratory functions and have a significant effect in declining infectious disease in children. Probiotics foods are consumed in different forms, mainly in fermented state and Kefir seeds are one such fermented product which contains symbiotic combination of Lactic acid bacteria and yeast. The consortium of lactic acid bacteria (LAB) present in kefir grains include *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Streptococcus* and yeasts (Cláudia *et al.*, 2012) and these microbiota are accepted as probiotic strains (Zhou *et al.*, 2009). Kefir grains are embedded with white or yellow irregular

granules of protein and a polysaccharide matrix called kefirin, produced by lactic acid bacteria (Hamet *et al.*, 2013). These peptides and exopolysaccharides synthesized during fermentation process are dominant in bioactive properties and reported to have anticarcinogenic, antimutagenic, antiviral and antifungal properties. The kefir grains can be used to ferment milk, cheese-whey or fruit juice, molasses or sugary solution (Dias *et al.*, 2007). The development of fruit juice-based fermented beverage with kefir seeds will be desired by consumers as healthy drink.

In the present study, Jamun fruit (*Eugenia jambolana*) was selected for fermented process using kefir seeds. Jamun fruits are highly nutritious, antioxidant rich (Debjit *et al.*, 2013) and reported to have lots of medicinal properties (Kumar *et al.*, 2008). Ayurveda and Unani Indian medicines have recommend Jamun fruit for several health problems such as digestive disorders, diabetes, liver problems and skin ailments and it acts as good blood purifier [Veigas *et al.*, 2007]. The fruits are utilized in preparation of juices, squash, Ready to Serve (RTS) beverages, jam and jelly

(Sonawane and Arya, 2013). To enhance the dual benefit of both probiotic and Jamun fruit, the present research aims in formulating Probiotic Jamun squash (PJS) using kefir seeds. The dominant probiotic microbiota present in kefir grains was evaluated. Nutraceutical analysis such as nutritional, antioxidant, antimicrobial activity and anti-diabetic was elucidated.

Materials and Methods

Physio-chemical properties of jamun fruit

Physio-chemical properties of whole fruit such as weight, length, firmness, shape index and colour were identified (Shahnawaz and Shiekh, 2011). Consumable part (pulp) and non-consumable (seed) matters were separated and evaluated. Juice yield was evaluated through obtaining fresh juice from 100gm of selected Jamun fruits and other physio-chemical parameters such as pH, titrable acidity and total soluble solids (TSS) were also analysed. The pH of the sample was measured using digital pH meter. TSS was analysed using hand refractometer and total acidity in Jamun juice was determined by titrating with 0.1N NaOH previously standardized using standard oxalic acid and the values were expressed as tartaric acid equivalents.

Production and Analysis Kefir grains

The commercial dehydrated Kefir seeds were bought from Malaysian biocare shop, Malaysia. The obtained seeds were rehydrated using pasteurized milk. The seeds were inoculated into the milk and were allowed to ferment for about 24 hours. After fermentation period, the seeds were removed by filtration. The procedure was followed for one week period until necessary quantity of Kefir seeds were obtained (Farnworth, 2005). It was stored at refrigerated (4°C) temperature for further use. Lactic acid bacteria present in the kefir seeds was evaluated through serial dilution and sub cultured in MRS (deMan, Rogosa and Sharpe) agar medium. Plates were observed for individual colonies and triplicates were maintained. Biochemical tests such as Gram staining, Indole test, Catalase test, Casein hydrolysis test, motility, Methyl Red (MR) test, Voges Proskauer (VP) test and carbohydrate fermentation test was performed to confirm the presence of probiotic bacteria.

Preparation of Jamun Squash

Jamun squash was prepared according to method outlined by (Syed *et al.*, 2012). Fruits were washed and dried to remove the water content. The fruit juice was prepared through juice extractor. Sugar syrup was prepared by boiling required quantity of water, sugar and citric acid. The recipe of the Jamun squash includes, 250mL of pasteurized strained juice mixed

with 750 mL of sugar syrup. The content was filled into sterile bottles and stored at refrigerated temperature (4°C) for further analysis.

Probiotication of Jamun squash using kefir grains

Kefir grain of 0.4 g (dry weight) was dispensed into 500mL of pre-sterilized Jamun squash followed by incubation at 10°C and 25°C for 24, 48, 72 hrs and it was refrigerated at 4°C temperature for further analysis (Mousavi *et al.*, 2011).

Sensory evaluation

The sensory evaluation of the probioticated Jamun squash was carried using 9-point Hedonic scale score (Puerari *et al.*, 2012). Drink was evaluated for acceptability with the help of pre-trained 20 panel members and they were asked to indicate the range of liked or disliked product on a 9-point hedonic scale (9=like extremely; 1=dislike extremely). The evaluation was made based upon four essential parameters namely colour, taste, odour and appearance.

Nutraceutical evaluation of JS and PJS

The nutraceutical properties such as nutritional analysis, total phenolic content, total flavonoid, antioxidant activity were evaluated for JS and PJS. The total sugar content was evaluated through Anthrone method (Hodge *et al.*, 1962). The absorbance was measured at 620nm against reagent blank and total sugar content was expressed as g/100 mL of glucose. The protein content was measured using Lowry's method (Lowry *et al.*, 1951). The cholesterol content was analysed through Zak's method (Shivaraja *et al.*, 2008) and Vitamin C content present in JS and PJS was measured (Ranganna, 1999). Total phenolic content (TPC) of samples/standard was determined using the Folin-Ciocalteu reagent method (Mohamed *et al.*, 2013). The absorbance of the reaction mixtures was measured at 650 nm. Gallic acid was used as a standard. Total flavonoid content (TFC) of samples/standard was determined using the aluminium chloride method (Barku *et al.*, 2013). Quercetin was used as the standard for the calibration curve. Antioxidant activity of the samples (Jamun squash, Kefir, Probiotic drink) was analyzed by DPPH assay (Blois, 1958).

Antibacterial activity

The antibacterial activity of the samples (JS, Kefir, PJS) was evaluated using well diffusion method (Nair *et al.*, 2005). Four bacterial strains such as *Escherichia coli* (MTCC 1692), *Staphylococcus aureus* (MTCC 7443), *Klebsiella pneumoniae* (MTCC 7407) and

Salmonella typhi (MTCC 733) were obtained from the Microbial Type Culture Collection (MTCC). Test organisms were spread onto the Muller Hinton Agar (MHA) medium. 50µL of samples were loaded into 5 mm well made on the inoculated agar. The inoculated plates were incubated for 24 h at 37°C. Ampicillin, gentamicin and tetracycline were used as positive controls. The diameters of inhibition zones were used as a measure of antibacterial activity. All determinations were performed in triplicate and the mean values are noted.

Antidiabetic activity

The α -amylase inhibition disk assay was performed (Apostolidis *et al.*, 2006). 800µL of all the three samples such as Jamun juice, Kefir, Probiotic Jamun juice was taken and 200µL of α -amylase solution was added to each samples. 100 µL of this mixture was added to sterile paper disk and it was then placed in Petri plates containing starch agar medium with control as paper disk containing only the α -amylase solution. The plates were incubated at room temperature for 24 hours. After incubation, 5 mL of iodine stain was added and kept aside for 15 minutes to obtain the zone of inhibition. The percentage of α -amylase inhibition was calculated using the following equation

$$\% \text{ of } \alpha - \text{amylase inhibition} = \frac{\text{Diameter of control} - \text{Diameter of sample}}{\text{Diameter of control}} \times 100$$

Shelf life analysis

Shelf life analysis for the formulated probiotic Jamun squash was performed (Nosrati *et al.*, 2014). Sample was taken at weekly intervals, and viability of LAB in probioticated Jamun squash was determined and expressed as colony forming unit per mL of solution (CFU/mL).

RESULTS AND DISCUSSION

Physio-chemical properties of Jamun fruit

The results of physio-chemical properties of Jamun fruits were illustrated in the Table 1. The average weight of Jamun fruit was observed to be 7.53gm and length was found to be 3.43cm. The selected fruits were soft in firmness. Moreover, the shape and colour was found to be elliptical and blackish purple respectively. The edible (pulp) and non-edible (seed) portion of selected Jamun fruit was found to be 68.16% and 31.83% respectively and whereas, quantity of juice obtained from 100g of fruits was found to be 41.6mL. Analysis of above mentioned parameters helps in sorting out similar quality of fruits

without deviation which will enhance the juice processing. Analysis of physical properties can be used to designing and sizing machine components in pre-operative process (Stroshine, 1998). The fresh extract of the Jamun fruit was found to be acidic in nature with pH value of 3.6 and acidity of 1.36%. Hence selected Jamun fruit are acceptable for formulation of squash with improved shelf-life. According to Shahnawaz and Shiekh (2011) pH of fruits correlates with astringency taste and naturally acidic fruits retain better shelf life. Hasnain and Ali (1990) have stated the similar view that lower pH fruits are highly favourable for improved shelf-life.

Morphological and biochemical characterization of LAB isolated from Kefir grains

Volume of the kefir seeds increases with continual sub-culturing and the presence of Lactic acid bacteria in the Kefir seeds were confirmed through morphological and biochemical characterization (Table 2). A total of three different bacterial strains were identified with morphological variations. All the strains were Gram positive and non-motile. Similarly, indole test and casein hydrolysis gave positive results. KSRK03 strain was characterized with colony morphological difference of cocci shape with pale yellow colour, glossy and smooth border. Moreover, KSRK05 was identified as rod and spherical shape with white colonies and smooth border and whereas, KSRK06 strain was morphology identified with single and cluster short rod shape, small colonies with smooth end. Other biochemical test such as indole, catalase, MR-VP test showed negative results. The results of carbohydrate utilization test revealed that all the isolates were able to ferment glucose, fructose, lactose and sucrose.

Hence, the principal characteristics of bacterial genus were confirmed to be probiotic strain of *Lactobacillus* spp. The present study correlates with the work done (Franzetti *et al.*, 1998) who have identified the presence of *Lactobacillus casei* as a predominant strain in kefir seeds. It is also reported a consortium of LAB along with yeast and few acetic bacteria were present in kefir seeds (Gulitz *et al.*, 2011). Hence, the above results evidently illustrate that kefir seed contain consortium of probiotic bacteria and it can be used as seed culture for the probiotication of Jamun squash.

Sensory evaluation of PJS

The sensory evaluation of PJS was evaluated through Nine-point Hedronic scale method (Puerari *et al.*, 2012) and results were illustrated in the Table 3. Among 20 respondents, fermented Jamun squash at 10°C during 72 hrs incubation gave best results of 90%

like, 5% of dislike and 5% of neither like or dislike. On the other hand, the Jamun squash fermented at 25°C with 48hrs and 72hrs gave lower acceptance level with like percentage of 55 and 60 respectively. The study conducted by Puerari *et al.*, (2012) gave similar results of best acceptance at 10°C for fermented cocoa pulp-based kefir beverages and reason might be due to mild fermentation process of microbial consortium present in kefir seeds. Moreover, lower concentration of acidity produced in the beverage at 10°C was highly accepted. The level of volatile compounds present in the end product depends upon the fermentation duration which reflects over sensory quality (Puerari *et al.*, 2012).

Nutraceutical evaluation of Jamun squash and probioticated Jamun squash

From the Table 4, it was observed that the total sugar content of the JS was found to be high (4.1 mg/mL) when compared to PJS. This may perhaps due to the conversion of sugar into organic acids during fermentation process. However, the protein content was high in probiotic drink (2.86 mg/mL). The cholesterol content was lower in PJS (451 µg/mL) and enriched with vitamin C content of 134 µg/mL which was significantly higher than compared to JS. This result was slightly similar to that of Muhammad *et al.* [28] who found that the Jamun squash contains 2.31% total carbohydrate, 0.69% protein and 0.05% fat and 4.47 mg vitamin C.

From the Fig.1, it was observed that Total Antioxidant Content (TAC) of JS and PJS was 1.2 and 1.56 mg AAE/mL respectively. Similar result was reported by Islam *et al.* 2013 using catechin as standard and the absorbance at 700 nm for crude methanolic extract of Jamun and it was found to be 1.90 mg AAE/mL. The result reported by Jayaprakasha *et al.* (2008) indicated that the TAC was due to the presence of phenolic, flavonoids and ascorbic acid. Total Phenolic Content (TPC) of JS and PJS was 5.1 and 5.8 mg GAE/mL respectively. These results were comparable with Sonawane and Arya (2013) who observed TPC of Jamun pulp i.e., 5.53 mg GAE/g of sample. Total Flavanoid Content (TFC) was determined in comparison with standard quercetin and the results are expressed in terms of mg QE/mL of sample. Total Flavanoid Content (TFC) of JS and PJS was 5.34 and 6.31 mg QE/mL respectively. This result is in accordance with the results of Mohamed *et al.*, (2013) who observed TFC in methanolic extract of Jamun was 6.22 mg QE/g of dry sample. The present result is in accordance with the report of Zheng *et al.*, (2011) who stated that the flavonoid content present in *Syzygium jambos* extracts was responsible for its antioxidant activity.

Antibacterial Activity of probioticated Jamun squash, Jamun squash and kefir seeds

The results of antibacterial activity of PJS, JS and kefir seeds against selected bacterial pathogen such as *E.coli*, *K. pneumonia*, *S.typhi* and *S.aureus* are presented in the Table 5 and Fig. 2. All the samples exhibited zone of inhibition against both Gram positive and Gram negative microorganism. Among the tested samples, PJS showed the maximum inhibition for *K. pneumonia*, *S.typhi* and *S.aureus*. On the other hand, all the samples showed similar percentage of inhibition against *E.coli*. The zone of inhibition obtained for the sample is compared to three different standard antibiotic discs such as gentamycin, tetracyclin and ampicillin. This result was similar to that of Sirirat and Jelena, (2010) who found the antibacterial activity of kefir and obtained 10 mm and 12.5 mm of zone of inhibition for *S.aureus* and *E.coli* respectively. Also this result is in accordance with Mohamed *et al.*, (2013) in which *E.coli* and *S.aureus* shows 22 and 24 mm inhibition respectively against methanolic extract of Jamun. Zubillaga *et al.*, (2001) indicated that the factors responsible for the killing of pathogenic microbes may be due to the presence of bioactive compounds such as bacteriocin, hydrogen peroxide and organic acids.

α -Amylase Inhibition Assay

The results of α-amylase inhibition assay were represented in the Table 6 and Fig. 3. The probiotic Jamun juice extract possess lower zone of inhibition (12.62 mm) which indicates that the conversion of starch to glucose was reduced when compared to control which possess 24 mm of inhibition zone. The results of percentage of α -amylase inhibition of probiotic Jamun juice extract was highly significant with 47% when compared to Jamun juice extract and kefir seeds of 40 and 38% respectively. Thus the probiotic juice helps in reducing the glucose production. This result is in accordance with Alam *et al.*, (2012) who indicated the presence of Lupeol, 12-oleanan-3-ol-3β-acetate, stigmasterol, β-sitosterol in n-hexane portion of Jamun by NMR analysis which has significant effect on lowering serum glucose concentration with a concomitant increase in insulin level indicating it's hypoglycemic and insulin stimulatory activity. Further the result provided by Judiano (2012) revealed that kefir prevents against glucotoxicity and lipotoxicity and reduces the occurrence of hyperglycaemia, associated with synergistic bioactive compound found in kefir themselves. Hence this may the reason for reduction in cleavage of starch to glucose molecule with the aid of α-amylase.

Table 1. Physio-chemical properties of whole fruit, extracted pulp and juice of Jamun fruit

Sr. No	Parameters	Inference
1.	Weight (gm)	7.53±0.85
2.	Length (cm)	3.43±0.20
3.	Firmness	Soft
4.	Shape index	Elliptical
5.	Colour	Blackish-purple
6.	Edible portion – pulp (%)	68.16±2.84
7.	Non Edible portion seed (%)	31.83±2.84
8.	Juice yield (mL)/100gm of fruit	41.6±1.52
9.	pH	3.6±0.1
10.	Total Soluble Solid (TSS)(°B)	15.6±1.12
11.	Acidity (%)	1.36±0.15

Values were means ± SD

Table 2. Morphological and Biochemical characterization of Lactic acid Bacteria present in Kefir grains

Biochemical test	KSRK03	KSRK05	KSRK06
Colony morphology	Pale yellow with glossy and smooth border	White colonies with smooth border	Small colonies with smooth ends
Spore formation	Negative	Negative	Negative
Gram staining	Gram positive Cocci	Gram positive Rod & spherical	Short rod with single and cluster
Motility test	Non motile	Non motile	Non motile
Indole test	+	+	+
Catalase test	-	-	-
Casein hydrolysis test	+	+	+
Citrate fermentation	+	-	-
Methyl Red (MR) test	-	-	-
Voges Proskauer (VP) test	-	-	-
Glucose fermentation	+	+	+
Fructose fermentation	+	+	+
Lactose fermentation	+	+	+
Sucrose fermentation	+	+	+

KSRK03, KSRK05 and KSRK06 – Designation of bacterial cultures isolated from Kefir grains.

Table 3. Sensory evaluation of Probiotic Jamun squash

Description	Scale point Assigned values	No. of responses					
		Probioticated Jamun squash					
		@10°C			@25°C		
		24h	48h	72h	24h	48h	72h
Like extremely	9	1	1	3	1	1	2
Like very much	8	6	5	7	4	4	3
Like moderately	7	6	7	4	3	2	3
Like Slightly	6	1	3	4	3	4	4
Neither like or dislike	5	2	1	1	2	3	4
Dislike Slightly	4	1	1	1	2	0	0
Dislike moderately	3	2	1	0	2	3	3
Dislike Very much	2	1	1	0	2	3	1
Dislike extremely	1	0	0	0	1	0	0
No. of respondents		20	20	20	20	20	20
SD±		2.22	2.33	2.44	0.97	1.56	1.56
Dislike %		20	15	5	35	30	20
Like %		70	80	90	55	55	60
Neither like or dislike%		10	5	5	10	15	20

Table 4. Nutritional analysis of Jamun Squash and probioticated Jamun Squash

Parameters	Jamun Squash	Probioticated Jamun Squash
Total sugar (mg/ mL)	4.1±0.15	2.2±0.17
Protein (mg/ mL)	2.4±0.10	2.86±0.05
Cholesterol (µg/mL)	856.3±3.2	451±1.0
Vitamin C (µg/mL)	25.3±0.57	134±1.0

Values were means ± SD

Table 5. Antimicrobial activity of Jamun squash, kefir and probioticated Jamun squash

Strain name	Zone of inhibition (mm)					
	Gen	Tet	Amp	JS	Kefir	PJS
<i>E.coli</i>	7	19	-	16.33±1.14	16.66±1.52	17.66±1.527
<i>K.pneumoniae</i>	22	25	10	16.33±1.00	17.66±1.52	20.33±1.52
<i>S.typhi</i>	21	24	12	10.66±0.57	12.33±1.00	20.66±1.52
<i>S.aureus</i>	7	19	-	10.33±1.52	14.66±0.577	18.33±1.00

Shelf life study

The probioticated Jamun squash was tested for shelf life study. The viable cell counts were remarkably dropped during the storage (Fig.4). The initial total viable count of the probiotic drink was 2.4×10^7 CFU/mL which decreased to 1.3×10^7 at refrigerated storage. Although the viability of the population of LAB decreased, the viable count of the probiotic drink did not fall below 10^6 CFU/mL. The result is in accordance with Divya and Kumari (2009) reported a decline in total viable count of *Lactobacillus reuteri* and *Biofidobacterium bifidum* of whey based probiotic beverage stored at $4 \pm 1^\circ\text{C}$.

Table 6. α -Amylase inhibitory activities of Jamun fruit juice extract, kefir and probioticated Jamun fruit juice extract

Samples	Zone of inhibition (mm)
Jamun fruit juice extract	14.33±0.57
Kefir	15.00±0.32
Probiotic Jamun fruit juice extract	12.62±1.52
Control	24

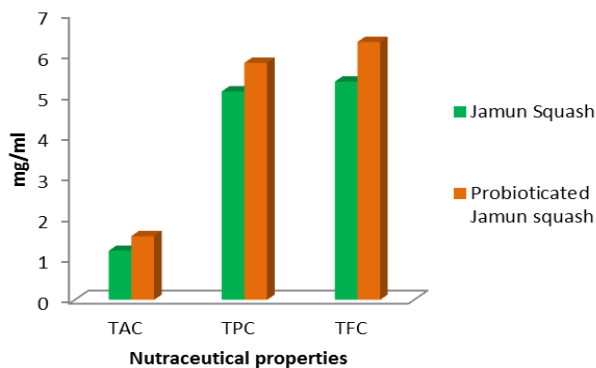


Fig 1. Nutraceutical properties of Jamun squash and Probioticated Jamun squash.

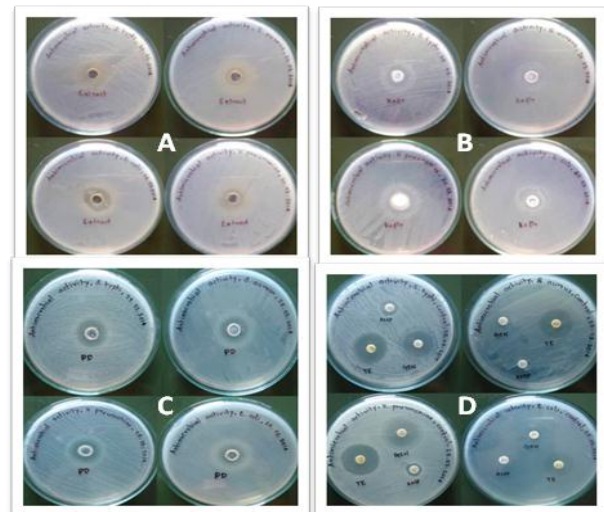


Fig. 2. Antibacterial activity of selected samples against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Salmonella typhi* (Samples : (A) JS (B) kefir (C) PJS (d) Antibiotic disc (Gentamycin, Tetracyclin and Ampicillin).

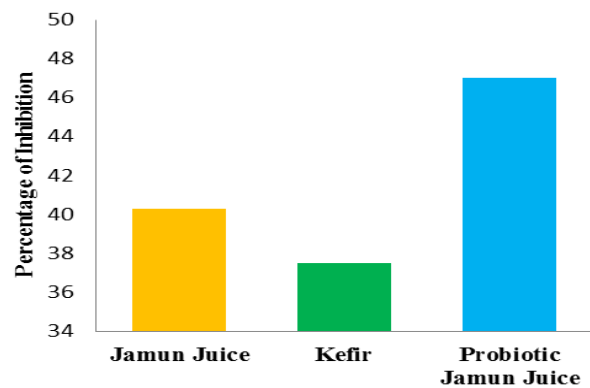


Fig. 3. α - amylase inhibition assay of Jamun juice, kefir and probioticated Jamun juice

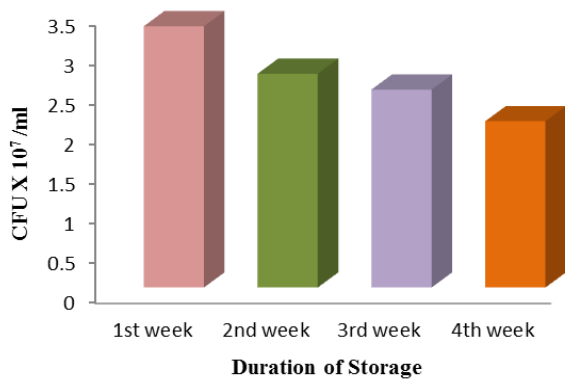


Fig. 4. Shelf life of Probioticated Jamun squash

CONCLUSION

The present research clearly elucidates that PJS acts as promising carrier for survival of probiotic bacteria which in turn have dual nutraceutical benefits. Lactic acid bacteria present in the kefir seeds are capable of utilizing the nutrients present in the Jamun juice and able to proliferate. Moreover, various physio-chemical parameters such pH, TSS and acidity were found to be favourable for fermentation process carried out by microbiota present in kefir seeds. The microbial analysis of kefir seeds exhibited the presence of probiotic bacteria and three strains KSRK03, KSRK05 and KSRK06 isolated and identified. Moreover, the microbiota depends on source of kefir grain used. PJS has exhibited greater improvement protein and vitamin C content than compared to JS with significant difference of 2.86 mg/mL and 134 μ g/mL respectively. Moreover, Total antioxidant, total phenolic content, and total flavonoid content were enriched in PJS through probiotication process. The results of antimicrobial activity of PJS gave favourable results with significant antagonist activity against *K.pneumonia* and *S.typhii*. Antidiabetic activity was found to be superior for PJS with percentage of inhibition of 47% when compared to kefir seeds and Jamun juice. The present study highlight the beneficial effect of Jamun squash fermented with kefir seeds had an acceptance level of about 90% for fermentation period of 72hrs at 10°C. Survival of probiotic bacteria in Jamun squash has gradually reduced and found to at approved level of 1.3X10⁷cfu/mL at the end of fourth week at refrigerated temperature. Hence from above research it can be concluded that kefir seeds can be used as starter culture for fermentation of fruit drinks and it can be given as a probiotic supplement.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Factors affecting on mortality rate in the broiler chicken production farms in Erbil, Iraq

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ABSTRACT

Poultry meat have become the main source of animal protein in the human diet in Iraq and in the past few years poultry production has become one of the most dynamic agricultural subsectors in the governorate of Erbil in particular. It's important to identify the problems and constraints of the poultry sector, especially on broiler chicken production. The purpose of this paper is to provide regional and local agricultural authorities with current basic information on the poultry farms and production inputs for use in policy formulation and making business decisions. Also determine the effects of factors on the mortality rate in broiler farms in Erbil province. This study depend mainly on data collected from 180 broiler farms during 2015 by a questionnaire method. It has been designed by select a random sample of farms from all regions of Erbil. The results indicated that the four independent variables (Farm capacity (bird), number of workers, consumed feed (ton), production (tone)) in all rotations (1 to 4) have a strong significant relationship with the dependent variable of mortality. Moreover, education of farmers has a significant effects on mortality in the first and second rotation but it does not have a significant relationship in the third and fourth rotation. On the other hand, age of farmers and experience do not have a significant relationship with the mortality variable in each rotation.

Keywords: Broiler chicken farm, Factors, Mortality, Erbil-Iraq

INTRODUCTION

Agriculture has the probable to again be a dynamic contributor to the economy of the Erbil Region and to the economy of Iraq as a whole. The agriculture strip contribution to the national economy is second only to that of the oil sector. Agriculture can serve as the foundation to poverty reduction and economic stability (Natali, 2010). Today focused broiler production cannot be conceived without the thorough knowledge of the affecting determinant factors and the effective applications of these (Zoltán, 2010). Poultry meat is an important source of high quality protein, minerals and vitamins to balance the human diet (Bandara, et al., 2006).

Poultry industry occupies great importance not only in Iraq's economy, but in most of the world economies as they provide essential food items to citizens because of the protein in eggs and chicken meat, and in Iraq increasingly important addition to what has been reported as running to tens of thousands of unemployed people by virtue of the fact that most of the production projects Poultry in Iraq is small and spread out projects in most provinces of Iraq (Daghir, 2008). Modern poultry production in Erbil began in the middle 1970's with the establishment of a few devoted poultry farms. Poultry production has expanded so that today there are 100

of farms scattered throughout the governorate. In the wake of the Gulf War in the early 1990's, the poultry subsector persevered destruction and loot, and production crumpled to less than 10 percent. Amid the other portion of the 1990's, the subsector had a revival in Erbil thanks to the political stability in this region. However, this recovery was made possible by the procurement of highly subsidized production inputs and restricted markets. In 2003 the poultry subsector suffered from the disruptions brought on by the removal of subsidies in feeds, veterinary supplies, and gear, and breakdowns in the supply of power (USAID, 2005).

Erbil is suffering from the problem of providing food, to provide an appropriate level of self-sufficiency in all food commodities, therefore the state focused on poultry production sector (USAID, 2005). Increased investments in poultry due to its high yield and speed of capital turnover industry projects, and lower the costs of obtaining the unity of the protein. The Northern Region of Iraq, especially the province of Erbil is facing the problem of food insufficiency of agricultural production, plant and animal to meet the growing needs of citizens which led and forced the country to import substantial amounts of agricultural products to meet demand (Baban and Serwan, 2014).

There are many studies have been carried out to increase the broiler production in the world. The usage of pads on the effectiveness of broiler production and poultry place preference and its effects (Sarica and Çam, 1998; Tumer, 2013), free breeding system and its effects and the cost of production (Şekeroğlu and Diktas, 2012), the density of the poultry (population) (Kaynak, 2010), the mortality incidents during the transportation of the chickens (Voslářová, 2007), factors affecting profits of broiler enterprises (Cevger and Yalçin, 2003). This study attempts to fill this void by analyzing effects of factors on the mortality rate in the broiler chicken farms in Erbil- Iraq. It's important to identify the problems and constraints of the poultry sector, especially on broiler chicken production. The objective of this paper is to provide regional and local agricultural authorities with current basic information on the poultry farms and production inputs for use in policy formulation and making business decisions. Also determine the effects of factors on the mortality rate in broiler farms in Erbil province.

MATERIAL AND METHOD

To obtain the necessary data for the study, a questionnaire survey has been designed particularly for this purpose collected through the poultry producer's dealers with traders of province Erbil in Iraq during 2015. The main purpose to choose the governorate of Erbil being the top provinces of the Northern Region of Iraq in terms of the number of broiler chickens fields, and its better representation of our purpose. But one of the objectives of this study was to assess the existing production system of poultry in Erbil districts. However, in an empirical investigation, it is impossible to collect information from the whole population.

Therefore, the researchers are often forced to make inferences based on information derived from a representative sample of the population. The sample size and the degree of variation usually affect the quantity and quality of information obtained from the survey; easy to provide unbiased estimates with small 'variance'. The independent variables chosen for the paper are age, education level and experience of farmers, capacity of farm, number of workers, consumed feed (ton) and production. The rotations mainly during one year, that's mean rotation one in winter, rotation two in fall time, rotation three in summer time and rotation four in spring time. Data were analyzed by using t and F test in SPSS software to finding the result of effect value on mortality in broiler chicken farm in four different rotations. The

following four tables contain a description and analysis of the variable.

RESULT AND DISSCUTION

Socio demographic characteristics of broiler farmers are shown in Table 1. The gender profile of the producers is showed that 100% of the broiler producers participating in the questionnaire is male. The data on age group of the sampled poultry farmers age profile of the producers. The age distribution of farmers indicated that 28.9% were less than 35 year of age. The age range of 36-49 year was 55.6%. And third group of age was 15.6.5% were above 50 year of age. The categories used were Illiterate level, primary, secondary, educated and other, Information about education of selected poultry farmers was analyzed and found and the result showed Illiterate was 8%, and 18% had complete primary education, 32% did their secondary education, while 23% had vocational qualification, 15% was agronomist education, and 4% was veterinarian.

The year of experience result showed that 21.7% of the respondent farmers are having poultry experience less than 5 year of experience, the rages year of experience was between 6-15 was 56.7%, and 21.7% having experience above 16 year. And capacity of farm we divided the farms into three size groups, as follows: less than 12,000 birds 12,000 to 20,000 birds' more than 20,000 birds. Frequency distribution of the registered farms according to farm size, less than 12,000 bird was 44.4%, between 12,000 to 20,000 was 41.1%, and more than 20,000 was 14.4%. Worker in broiler farm play a important rule for running the farm. The result showed that 63% used less than 3 workers, while 24% has between 4 to 5 workers, and 13% used more than 6 worker.

The consumed feed and production in each table are different in frequency and percentage that each Rotation have his table (Table 1 - R 1), (Table 2 - R 2), (Table 3 - R 3) and (Table 4 - R 4). The Consumed Feed in 4 rotations (R:1,2,3,4) result showed that in R1: 16.1%, R2: 17.8%, R3: 17.2%, R4: 13.9% of the respondent farmers are consumed feed less than 40 ton, the rages between 41-59 was R1: 37.8%, R2: 40.0%, R3: 39.4%, R4: 43.3%, and more than 60 ton consumed feed was R1: 46.1%, R2: 42.2%, R3: 43.3%, R4, 42.8%. The production of meat in farm was by ton in four Rotation, result showed that R1: 20.6%, R2: 25%, R3: 23.9%, R4: 17.2% of producer was less than 20 ton, the rages between 21- 49 was R1: 63.3%, R2: 63.3%, R3: 63.9%, R4: 71.1%, and production was more than 50

Table 1. Distribution of different broiler farmer's characteristics

Variable	Frequency	Percentage %
Age		
Less than 35	52	28.9
36 - 49	100	55.6
More than 50	28	15.6
Total	180	100.00
Education Level		
Illiterate	16	8.9
Primary	33	18.3
Secondary	58	32.2
Vocational	41	22.8
Agronomist	27	15
Veterinarian	5	2.8
Total	180	100.00
Experience Year		
Less than 5	39	21.7
6 - 15	102	56.7
More than 16	39	21.7
Total	180	100.00
Capacity of farm		
Less than 12000	80	44.4
12000 - 20000	74	41.1
More than 20000	26	14.4
Total	180	100.00
Total Worker		
Less than 3	112	62.2
4 - 5	45	25
More than 6	23	12.8
Total	180	100

Table 2. Distribution of different characteristics has 4 rotations

Variable	Rotation 1		Rotation 2		Rotation 3		Rotation 4	
	Frequency	%	Frequency	%	Frequency	%	Frequency	%
Consumed Feed								
Less than 40	29	16.1	32	17.8	31	17.2	25	13.9
41 - 59	68	37.8	72	40.0	71	39.4	78	43.3
More than 60	83	46.1	76	42.2	78	43.3	77	42.8
Total	180	100	180	100	180	100	180	100
Production								
Less than 20	37	20.6	45	25.0	43	23.9	31	17.2
21 - 49	114	63.3	114	63.3	115	63.9	128	71.1
More than 50	29	16.1	21	11.7	22	12.2	21	11.7
Total	180	100	180	100	180	100	180	100

Table 3. Result for different variable for Rotation 1 (winter time)

Variables		Means for mortality	Type of Test	P-value
Age (year)	Less than 35	2092.13	F test= 1.127	0.326
	36 – 49	2085.60		
	More than 50	2656.07		
Total		2176.23		
Education	Illiterate	2599.06	F test= 0.999	0.420
	Primary	2165.45		
	Secondary	2052.86		
	Vocational	2389.76		
	Agronomist	1693.70		
	Veterinarian	3180.00		
Total		2176.23		
Experience (year)	Less than 10	2091.47	t test = -0.738	0.462
	More than 11	2297.64		
Capacity Farm(bird)	Less than 12000	1501.13	F test= 22.308	0.000
	12000 - 20000	2049.20		
	More than 20000	3565.36		
Total		2176.23		
No. Worker	Less than 3	1655.45	t test = -4.633	0.000
	More than 4	3033.99		
Consumed Feed (ton) Rotation 1	Less than 40	1700.00	F test= 8.133	0.000
	41 – 59	1676.25		
	More than 60	2752.24		
Total		2176.23		
Production Rotation 1 (tone)	Less than 20	2729.73	F test= 11.087	0.000
	21 – 49	1724.96		
	More than 50	3244.00		
Total		2176.23		

Table 4. Result for different variables of Rotation 2 (fall time)

Variables		Mortality	Type of Test	P-value
		Mean		
Age (year)	Less than 35	2451.92	F test= 0.636	0.531
	36 – 49	2127.60		
	More than 50	2194.64		
Total		2231.72		
Education	Illiterate	2818.75	F test= 1.193	0.314
	Primary	2429.39		
	Secondary	2080.69		
	Vocational	1916.10		
	Agronomist	2266.67		
	Veterinarian	3200.00		
Total		2231.72		
Experience (year)	Less than 10	2300.94	t test = 0.657	0.512
	More than 11	2132.57		
Capacity Farm(bird)	Less than 12000	1697.88	F test= 16.881	0.000
	12000 - 20000	2092.86		
	More than 20000	3379.09		
Total		2231.72		
No. Worker	Less than 3	1843.13	t test = -3.498	0.001
	More than 4	2871.76		
Consumed Feed (ton) Rotation 2	Less than 40	2085.63	F test= 3.321	0.038
	41 – 59	1908.61		
	More than 60	2599.34		
Total		2231.72		
Production Rotation 2 (tone)	Less than 20	2519.78	F test= 4.339	0.014
	21 – 49	1975.18		
	More than 50	3007.14		
Total		2231.72		

Table 5. Result of different variables for in Rotation 3 (summer time)

Variables		Mortality	Type of Test	P-value
		Mean		
Age (year)	Less than 35	2296.40	F test= 1.415	0.246
	36 – 49	1973.16		
	More than 50	1839.29		
Total		2045.72		
Education	Illiterate	2165.63	F test= 2.417	0.038
	Primary	1933.33		
	Secondary	1902.22		
	Vocational	2215.85		
	Agronomist	1827.78		
	Veterinarian	3850.00		
Total		2045.72		
Experience (year)	Less than 10	1952.10	t test = -1.130	0.260
	More than 11	2179.81		
Capacity Farm(bird)	Less than 12000	1540.16	F test= 20.291	0.000
	12000 - 20000	2031.36		
	More than 20000	2983.18		
Total		2045.72		
No. Worker	Less than 3	1591.24	t test = -5.946	0.000
	More than 4	2794.26		
Consumed Feed (ton) Rotation 3	Less than 40	1838.71	F test= 6.069	0.003
	41 – 59	1717.08		
	More than 60	2427.13		
Total		2045.72		
Production Rotation 3 (tone)	Less than 20	1870.93	F test= 12.068	0.000
	21 – 49	1875.38		
	More than 50	3277.73		
Total		2045.72		

Table 6. Result of different variables for Rotation 4 (spring time)

Variables		Mortality Mean	Type of Test	P-value
Age (year)	Less than 35	2089.42	F test= 0.824	0.441
	36 – 49	1826.26		
	More than 50	1689.64		
Total		1881.03		
Education	Illiterate	1825.00	F test= 3.686	0.003
	Primary	2034.24		
	Secondary	1614.59		
	Vocational	2040.24		
	Agronomist	1609.63		
	Veterinarian	4300.00		
Total		1881.03		
Experience (year)	Less than 10	1865.75	t test = -0.166	0.868
	More than 11	1902.92		
Capacity Farm(bird)	Less than 12000	1385.50	F test= 21.366	0.000
	12000 - 20000	1715.29		
	More than 20000	2992.95		
Total		1881.03		
No. Worker	Less than 3	1472.29	t test = -4.437	0.000
	More than 4	2554.26		
Consumed Feed (ton) Rotation 4	Less than 40	1614.40	F test= 11.026	0.000
	41 – 59	1413.08		
	More than 60	2441.64		
Total		1881.03		
Ration production 4 (tons)	Less than 20	1817.74	F test= 13.503	0.000
	21 – 49	1656.53		
	More than 50	3342.86		
Total		1881.03		

ton that R1: 16.1%, R2: 11.7%, R3: 12.2%, R4: 11.7% showed in Table 2.

We can notice that the significant effect values for the seven independent variables on the Mortality Rate, as shown in the above tables and thinking in any P-value has a greater than 0.05 or less than 0.05). We note the last four variables (Capacity Farm (bird), No. of Workers, Consumed Feed (ton), Production (tone)) in all rotations (1 to 4) have a strong significant relationship with the (Mortality), because the P-value for F-test equal to the zero is less than ($\alpha = 0.05$), also the mean difference for each variable not located between 95% confidence interval of the difference, about capacity farm different with research (Shittu, et al., 2014), same result with no. worker in (Yassin, et al., 2009) and different with Vale, et al., (2010).

That's mean all of these variables have a significant effect on mortality rate of broiler chicken farm in Erbil city. On the other hand, the two Age and Experience do not have a significant relationship with the mortality variable in each rotation (1 to 4), because the P-value is greater than $\alpha = 0.05$ and the mean difference for each variable located between 95% confidence interval of the difference, according to a research made in turkey have same result not significant (TUMER, 2013). That's mean these two variables do not have a significant effect on mortality rate of broiler chicken farm. Finally, the variable education has a significant relationship with the Mortality in the first and second rotation because the F-calculated in ANOVA Table is greater than F-tabulated, but it does not have a significant relationship with the Mortality in the third and fourth rotation because the F-calculated in ANOVA Table is less than F- tabulated and the P-value is greater than ($\alpha = 0.05$) same with research (TUMER, 2013) not significant. That's mean the variable (education) effects significantly on mortality rate of broiler chicken farm in the first and second rotation and it does not have significant effect on mortality rate of broiler chicken farm in the third and fourth rotation for Erbil city.

CONCLUSION

The importance of using t-test and ANOVA test through deciding and interpreting the significant effect values for the seven independent variables (Age, Education, Experience, Capacity Farm, No. of Worker, Consumed Feed and Production) on one dependent variable (Mortality Rate) for four seasons or rotations. We conclude that the four independent variables (Capacity Farm (bird), No. of Workers,

Consumed Feed (ton), Production (tone)) in all rotations (1 to 4) have a strong significant relationship with the dependent variable (Mortality) which can be seen by observing the P-value, that's mean all of these variables have a significant effect on mortality rate of broiler chicken farm in Erbil city. On the other hand, the two independent variables (Age and Experience) do not have a significant relationship with the mortality variable in each rotation. Finally, the independent variable (Education) has a significant relationship with the dependent variable (Mortality) in the first and second rotation, but it does not have a significant relationship with the dependent variable (Mortality) in the third and fourth rotation. This paper helps the policy and decision makers about contribution of farms of broiler chicken in north of Iraq to prediction and planning to the future in sensitive projects also to sustainable development especially for lower-income region. The importance of using multivariate statistical tools in dealing with the sensitive data, especially in the absence of accurate statistics on variables and factors affecting on the analysis of the broiler chicken production, based on accurate and objective indicators. Directing and building the capacity for worker families in the farms of broiler production to achieve its goals by using the best means and lower costs, within the limits of its financial resources and the facilities available.

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Current and emerging biomarkers in breast cancer and influence of diet on their activities

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ABSTRACT

The developments in the field of nutrigenomics and metabolomics have led to the discovery of biomarkers that are normally used for the early detection of cancer and efficacy of the cancer treatment. The statistics indicate breast cancer as the second important cause of death after cardiovascular diseases. Globally over 0.55 million deaths occur due to breast cancer where as in Pakistan one out of nine women encounter this menace at certain phase during the lifespan. The well-known risk factors for breast cancer are age, gender, family history, early menarche, overweight, smoking or exposure to smoke, night shift work, alcohol and abusive use of medicines and radiation therapy. Among these various factors diet, obesity, physical inactivity and tobacco use are modifiable. The most important one is early screening and diagnosis of cancer through biomarkers. Now-a-days the screening of the DNA and serum plasma is helpful to track cancer growth, development and reaction to therapy. Some of the most studied biomarkers and their potential to develop malignant in women are p53, HER2, BRCA1 & BRCA2, Ki-67 and Cyclin D-1. These are present on different chromosomes and alteration in them results in cancer. Influence of diet on these biomarkers also very important. The dietary interventions commonly used for management of cancer include plant-based proteins with every meal, low/moderate fat intake, minimum intake of processed and refined grain products, vitamin-mineral supplements and higher intake of fluids. In nutshell, balanced nourishment may lessen the occurrence and severity of breast cancer. In this review article, current scenario of breast cancer, risk factors, pathogenesis, diagnosis, current & emerging biomarker and influence of diet on these biomarkers has been briefly reviewed.

Key words: Breast cancer, risk factors, biomarker, dietary management, life style

INTRODUCTION

Breast carcinoma is an unrestrained development of cells in breast. It mostly occurs because of alterations in the genes which control or regulate the normal growth of the cells. A tumor can be benign or malignant. A benign tumor is not considered cancerous whereas malignant tumor is considered dangerous due to numerous mitotic activities and its invasion in surrounding and distant tissues (Danaei, 2009). Breast cancer is very serious health risk for women. According to estimation globally more than 0.55 million deaths occur due to this cancer (WHO, 2013) and has attained the status of 2nd most threatening cause of deaths after cardiovascular diseases (CVDs). Despite of advancements in the technology, its incidences have increased from 12% in 1990 to 15% in 2013 (IHME, 2016). In United States only, new cases of breast cancer and deaths were 0.23 million and 40,290, respectively (ACA, 2016). Pakistan has been ranked 7th worldwide with respect to breast cancer and is at the top amongst all Asian countries (WHO, 2014). However, little information is available regarding its occurrence and causative

factors in the country. In Pakistan one out of nine women develop this cancer at certain phase of their life (SKMCH, 2015). The prevalence rate of breast cancer in Pakistan is 50.1/100,000 women/year which is 2.5 times higher than neighboring countries (Yasmeen and Zaheer, 2014). Furthermore, it is estimated that every year more than 40,000 patients die due to breast cancer (Hayatin, 2012). World Health Organization (WHO) has proposed effective education, balanced diet and screening components as effective strategies to reduce death rates (12 to 37 lives per day in UK and USA). It has been established that 90% of breast cancer cases can be cured through early detection and treatment (Danaei, 2009).

Risk factors for cancers

Certain risk factors are associated with the development of breast cancer *e.g.* age of menarche, gender, family history, tobacco & alcohol use, obesity, lack of activity, drug abuse, lack of breast feeding, inappropriate dietary pattern, smoking, night shift work, radiation exposure, pesticide residues, use of

estrogen or progesterone derivatives for contraception or as a part of hormone replacement therapy regime, relation with of an ovarian cancer case family and dense breast tissues (Abdulkareem, 2013).

Pathogenesis of breast carcinoma

Breast cancer is caused by numerous predisposing and participating factors (Abdulkareem, 2013). The pathogenies route is given below (Fig. 1):

Diagnosis of breast cancer

As mentioned earlier, timely detection of breast cancer is helpful in its management and treatment. The following methods are mostly used for the screening and detection of breast cancer globally (ACA, 2016).

i) Medical history & physical exam

It the easiest way commonly used for early diagnosis of breast cancer. In this case, breast is physically examined using hand to find out presence of lumps. Every lump is not the indication of cancer. Proper examination of the lymph-nodes which are present above the collar-bone and in armpit are also necessary because the enlargement of these lymph-nodes give indication about the spread of cancer. For further confirmation visit to a qualified medical doctor is recommended.

ii) Mammograms

It is just like an X-ray or simply called, the x-ray of the breast. For this test, breast is gently placed between two plates for taking the x-ray pictures of breast to find out the spread of tissues in breast. This is very painful test; however, the gentle process is essential to produce a good and comprehensive mammogram. Benefit of this test is to provide the better image of tissues present in the suspected area of breast for proper diagnosis. Ability of this test to provide good results may depends on the density and size of tumor tissues. This test is also useful for patients with breast implantation, not for those younger than fifty years of age because these women have dense breast tissues which appear white on mammogram and make the detection of cancer so difficult.

iii) Breast ultrasound

It is also known as sonography and is useful to detect abnormalities in breast that can be felt by physical examination and not seen on mammography. It is the best way to find out if the abnormality is solid (such as a benign fibroadenoma or cancer) or fluid-filled (such as a benign cyst). It cannot determine whether a solid lump is cancerous, nor can it detect calcifications. Qualified health professionals recommend this

diagnostic test because it can measure the exact location and size of lump in suspected area of breast.

iv. Nipple discharge exam

This is also a very useful indication of breast cancer. If the discharge from breast is bloody or dark brown in color, then immediately visit to your lady doctor for further consultation. Sometimes the doctors recommend the ductogram test for proper examination of discharge. In this test very thin metal tube pass through nipple where the discharge is coming and collect this discharge in tube to check the cancer cells or infection.

v. Biopsy

It is also called conformational test and is done when physical assessment, ultrasound, mammograms, nipple discharge and other imaging tests detect abnormality in breast that may be cancer. It is the only method to express if cancer is actually present.it has two types:

1. Fine needle aspiration

In fine needle aspiration (FNA) biopsy, a very thin needle attached to a syringe is used to aspirate a lesser amount of tissue from a suspected area of breast and observed under microscope.

2. Surgical (open) biopsy

In this case breast is surgically opened to take out some tissues and examined under the microscope. It is very painful procedure and comparatively large size tissue sample is removed as compared to any type of needle biopsy. It is done in a hospital, with general anesthesia and is usually an outpatient procedure.

Biomarkers

A biomarker may be used to see how well the body responds to a treatment for a disease or condition (NCL, 2015).

Established biomarkers

1. p53 gene
2. Human epidermal growth factor receptor 2 (HEGR2)
3. BRCA1 & BRCA2

Emerging biomarkers

1. Ki-67
2. Cyclin D-1

Established biomarkers

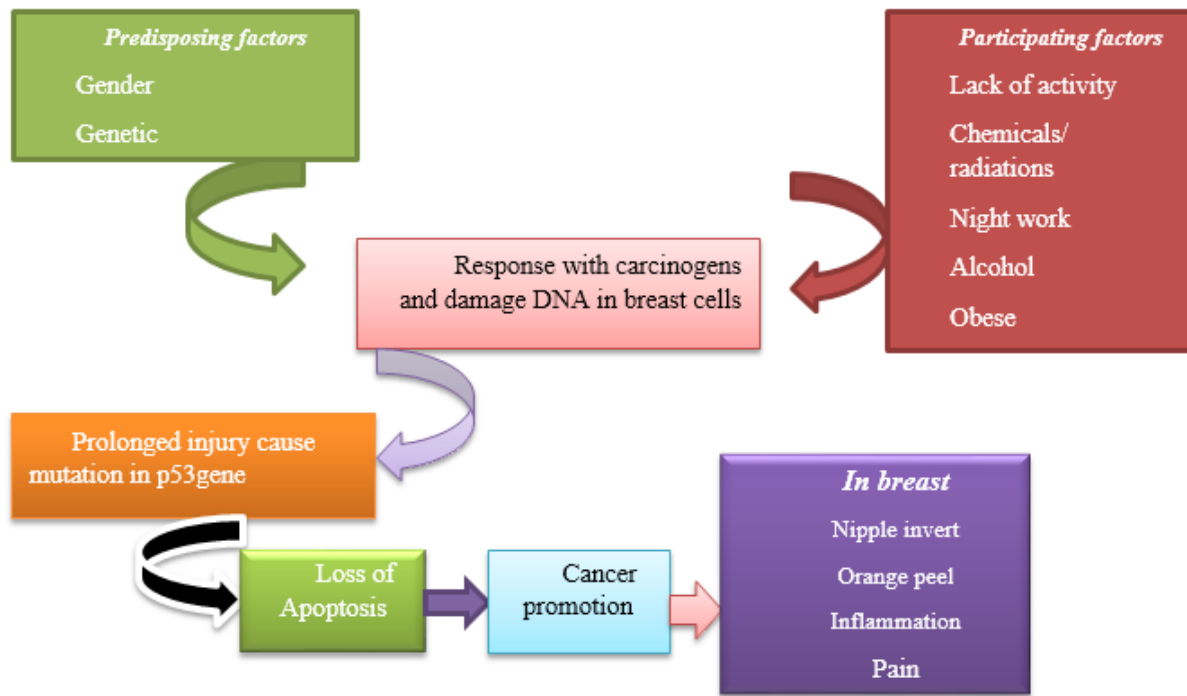


Fig. 1. Pathogenesis of breast cancer

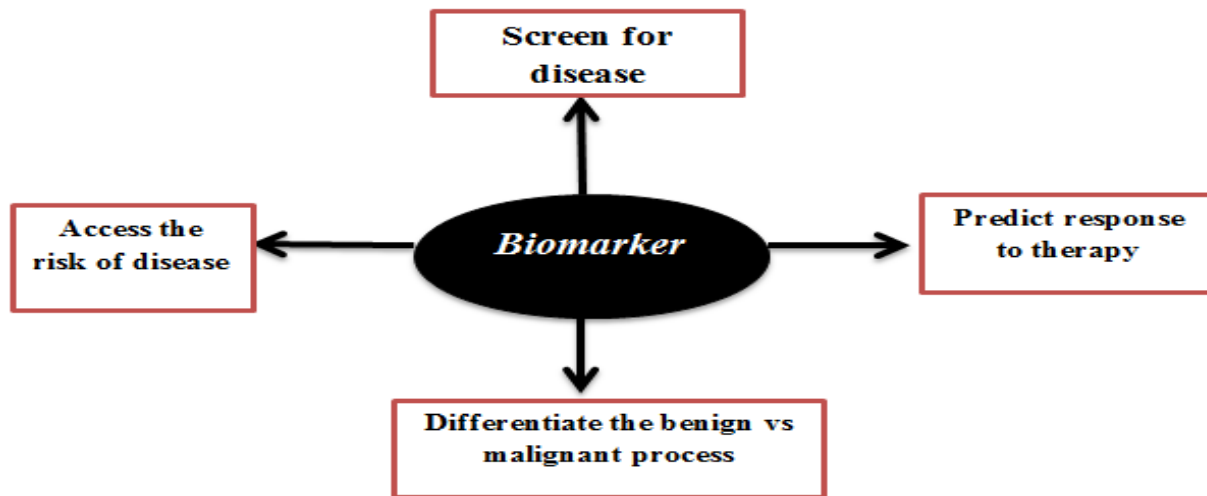


Fig. 2. Functions of biomarker

p53 gene

It is important biomarker of breast cancer and act as tumor suppressor gene or guardian gene. It is present on short arm of chromosome 17p and it act as a transcriptional factor to regulate the events of cell cycle (Lee *et al.*, 2011). If there is any stress or damage to DNA, it will activate and regulate the apoptosis but

when mutation occur in this gene then it cannot regulate the cell cycle and apoptosis not occur. It involve in transcription so called Tp53 name after the protein which it encode (Hosoda *et al.*, 2014).

Human epidermal growth factor receptor 2 (HEGR2)

It plays important role in healthy breast formation. It is glycoprotein and is present on long arm of

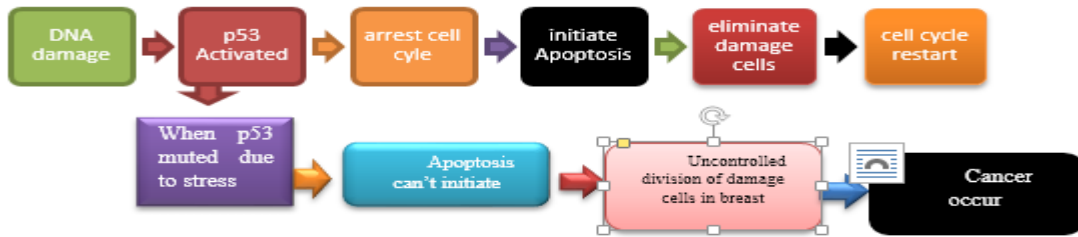


Fig. 3. Normal and muted process of p53 gene

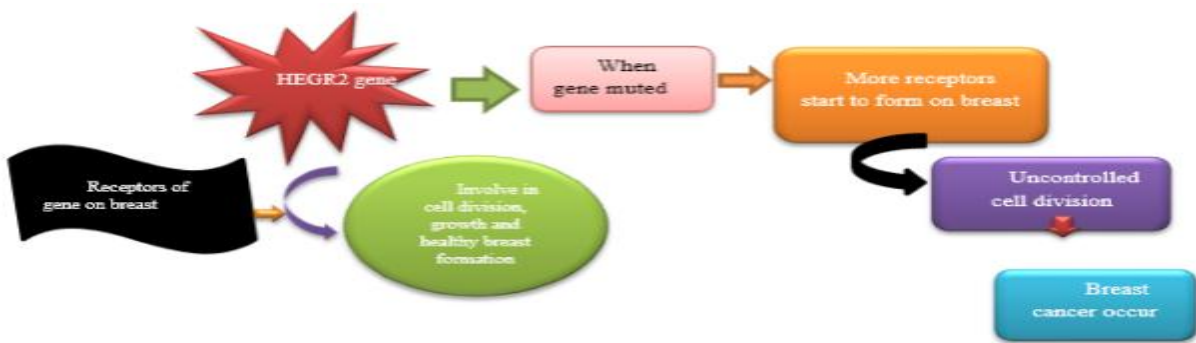


Fig. 4. Normal and muted process of HEGR2 gene

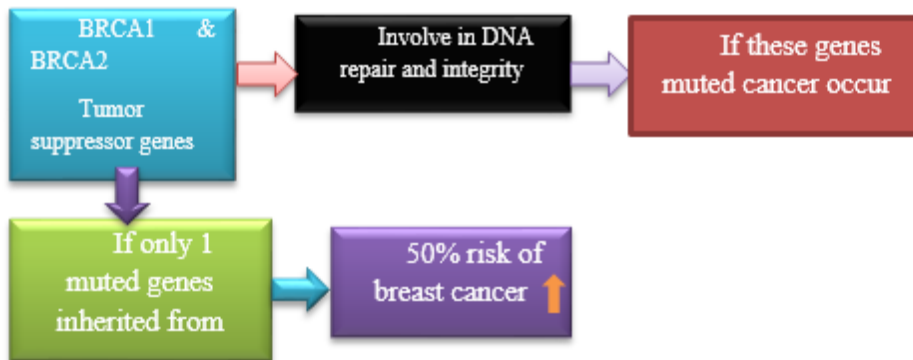


Fig. 5. Normal and muted process of BRACA1 & BRAC2 gene

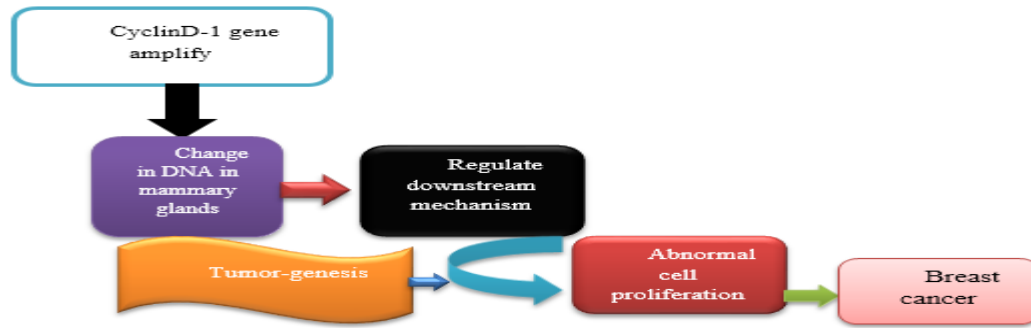


Fig. 6. Role of CyclinD-1 gene in breast cancer

chromosome 17q. Both genes p53 and HEGR2 are present on the same chromosome number 17 but differ in location *i.e.* one on short arm and other one on long arm so there is a strong association among these genes. It has been reported that 71.1% of patients suffering from HER2 positive breast cancer also contain mutated p53 (Misek and Kim, 2011). The receptors of HER2 are present on breast (number of receptors in normal cells are 20,000) which are mainly involved in the proper division of cell, health and growth of breast. As a result of mutation, gene sends messages to form more and more receptors on breast (number of receptors in muted cells are approximately 200,000). Consequently, uncontrolled cell division in breast leads to cancer (Arteaga *et al.*, 2014).

BRCA1&BRCA2

Both of these genes are present on chromosome 17q & 13q, respectively. These are basically tumor suppresser genes and are involved in genome integrity and DNA repair. Hereditary breast cancers mostly occur due to the mutation in these genes. Normally healthy BRCA1 & BRCA2 are essential to make proteins which keep the breast cell healthy and prevent them to grow in abnormal pattern. When congenital mutation occurs in these genes, the risk of breast cancer also increases. Up to 30% of breast cancer occurs due to mutation in these genes. At the age of 70, 80% of cancer risk is linked with BRCA1 and 50% with BRCA2. Muted gene in children is inherited by either from mother or father. Important thing to note that if only one gene is muted out of two, the chance of developing cancer become 50% in child (Felix *et al.*, 2014).

Emerging biomarkers

Ki-67

It is an emerging biomarker in breast cancer. It is basically a protein used as diagnostic tool for various

cancers. It is present on chromosome 10q and plays an important function in division and also essential for the normal process of growth. In breast cancer, it is used for identification of cell proliferating quantity (Langosch *et al.*, 2012). Its' expression varies in different phases of cell cycle; comparatively less in G1 and S phase (normal 6% but in cancer 10%), rapidly increased in mitosis (normal 10% but in cancer 20%) and then decreased in anaphase and telophase. When its rate or expression is beyond the normal range then its mean cell divide more rapidly and if untreated leads towards cancer. Ki-67 biomarker test is useful for early detection and treatment (Dowsett *et al.*, 2011).

Cyclin D1

It is also the emerging biomarker and present on the chromosome 11q and has been established as an oncogene. It is commonly over expressed and amplified gene in breast cancer. When it amplifies, cause changes in DNA of mammary gland cells and initiates the downstream mechanism which leads to tumor genesis of breast (Casimiro *et al.*, 2013).

Diet influence on biomarkers

Diet play important role in the enhancement of activities linked with biomarkers of breast cancer. However, different nutrients exhibit different effects on these biomarkers. For example, the higher availability of vitamin C increases the activity of p53 which regulate the cell cycle and increases apoptosis rate so more cancer cell start to eliminate or die (Kim *et al.*, 2012). Selenium-containing proteins also elevate the activity of p53 by interacting with them in the cells. Specifically, SeMet are important and when there is any damage to cell than these can activate p53 and assist the cell to repair its DNA. In such a way selenium may inhibit the development of cancer. Some foods also contain the harmful chemicals which set off the p53 like teas, coffees and "smoky flavoring" can destroy our DNA. These foods and flavorings

have chemicals like pyrogallol and gallic acid which are responsible for destruction of DNA and stop the p53 activity. Pyrogallol is mainly found in smoked foods, hair dyes, smoke of cigarette and coffee. Gallic acid is basic type of pyrogallol and is primarily found in tea and coffee (Manna *et al.*, 2009). Hormone melatonin is mostly responsible to enhance the activity of HEGR2 and is abundantly present in almonds, cherries, tomatoes, walnuts and oats. These have been shown to increase the rate of apoptosis and decrease the proliferation of HER2 breast cancer. On the other side if a person consumes too much red meat then there will be reduction of circulating melatonin. In diet, a high ratio of omega-3 to omega-6 polyunsaturated fatty acid is related to the decrease of risks of HER2 breast cancer. The combination of Docosahexaenoic acid (DHA) and curcumin is also effective in enhancing the rate of apoptosis and reduce the cancer risk (Ledesma, 2015). Likewise, there are numerous foods which have the ability to decrease the occurrence rate of breast cancer especially in BRCA1 & BRCA2 carriers. These foods include cabbage, cauliflower, broccoli, kale, soybeans, tomatoes and walnuts. According to a recent study, red meat consumption favourably amplified breast cancer risk in BRCA2 carriers. Likewise, in another study, increased risks were reported in heavy coffee drinkers who were BRCA1 carriers (Ledesma, 2015). Ki-67 is another important biomarker which is affected by the consumption of vegetables like cruciferous vegetables, onions and garlic which are good source of quercetin, resveratrol and insoluble dietary fiber. These compounds help to reduce the expression of Ki-67 gene by promoting apoptosis (Ledesma, 2015). There is little evidence about the influence of diet on Cyclin D-1 gene. However, low levels of mono-unsaturated fatty acids (MUFA) or unadjusted fat intake is associated with cyclin D1 overexpression and ultimately breast cancer (Kim *et al.*, 2011).

CONCLUSION

Scientific advancements in the field of health made it possible screening, early detection and prompt treatment of ailments once considered incurable. Breast cancer can be prevented by avoiding risk factors and is remediable if diagnosed early. The most studied biomarkers and their potential to develop malignant in women include p53, HER2, BRCA1 & BRCA2, Ki-67 and Cyclin D-1. The overexpression of mutant genes can be managed by taking plant based low fat diets alongwith higher intakes of vitamin-mineral supplements as well as fresh fruits and vegetable.

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Artificial sweeteners and their health effects

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ABSTRACT

Sugar plays an important role in our daily life. Sucrose is extracted from *Saccharum* spp and *Beta vulgaris* and is highly metabolically active and results in weight gain and type II diabetes. Due to these side effects companies launch various synthetic sweetening agents that have very low calories but are highly sweeter than normal table sugar, known as alternative sweeteners, artificial sweeteners, non-caloric sugars. These low nutrition synthetic compounds are part of soda drinks, chocolates, cakes and other baked products. Acesulfame-K, aspartame, neotame, saccharin, stevia and sucralose have been approved by the FDA for use in foods and/or drinks. People are moving toward artificial sweetener without being aware of their harmful effect. Many long term and short term studies have been performed regarding the deleterious effects of these artificial sweeteners on health. Frequent consumption of high-intensity sweeteners may dampens physiological responses, metabolic activities. The aim of this study is to pin point these long term as well as short term harmful effects on human health.

KEY WORDS: Artificial Sweeteners, Acesulfame-K, Aspartame, Neotame, Saccharin, Stevia, Sucralose, Metabolic activities

INTRODUCTION

There are various sweetening agent present naturally that enhance taste of everyday foods and have great nutritional importance. Sweeteners are either monosaccharide or disaccharide. Food industries also synthesize some chemicals that induce sweet taste but have no nutritional importance but are sweeter than natural sweetener. Natural sweeteners are extracted through honey, maple syrup, Molasses sans sucrose (Spillane, 2006). One of essential natural sweetener used in human diet is table sugar i.e. sucrose extracted mostly from *Saccharum* spp and *Beta vulgaris*. Harmful effects of table sugar on health provide a path to sugar alternatives. Millions of health conscious people worldwide consider sugar alternatives a healthy substance which is not true (Tandel, 2011). These sugar alternatives are known as artificial sweeteners (AS) or non-caloric artificial sweeteners (NCAS) and are abundant component of sugar free diet. The main reason behind use of these AS is because they are sweeter than table sugar, low in calories and cost, reduce weight and stabilize blood sugar level. Due to low cost and calories these NAS are commonly used in diet sodas, cereals sugar free desserts and also recommended for those patients that are suffering from type II diabetes (Gardner, 2012). Up-till now six AS are approved by FDA that acts as food additives i.e. Acesulfame-K, aspartame, neotame, saccharin, stevia and sucralose. Mostly NCAS doesn't digested in GI tract and interfere with

the working of microbiota i.e. microorganism that survive in human intestine and helps in production of vitamins and stabilization of GI tract activity, and result in metabolic syndrome. Health effects of only four of them are discussed below. The aim of this study is to identified pro and cons of chemistry, metabolism and toxicology of artificial sweeteners.

Factors important for sweeteners perception

The strength of taste development and perception depends on physical and chemical composition of medium in which sweetener is dispensed, sweeteners concentration, temperature and the pH of medium.

How sweetness can be enhanced?

Sweetness can be enhanced by making combination of more than two sweetening agents. Different sweeteners have different intensity, duration and after-taste, solubility and stability at different pH and temperature, so making combination will improve taste. NCAS when used in combination can increase sweetness; to reduce this sweet taste polyols (carbohydrates that are not sweet) are added. This combination of NCAS is preferred by companies because these NAS doesn't react with each other so they are safe to use. The caloric values for the polyols approved by FDA are from 1.6 to 3.0 depending type of polyols.

Combination of saccharin and aspartame is present in soft drinks, aspartame and acesulfame potassium in combination with sucralose are present in bottled drink. Combination of polyols and sweeteners is abundantly used in chewing-gums (Nabors, 2012).

How NCAS metabolized in body?

It is estimated via various studies that sweetened food are source of weight gain because they induce sensation of appetite, hence increase in food intake takes place. Both natural as well as artificial sweeteners stimulates production of taste by acting on lingual taste bud as well as sugar digesting molecule by acting on GLP-I releasing cell of intestinal mucosa (Jang et al., 2007). Variation in GLP-I secretion and gastric emptying was observed in those young healthy adult who consume diet soda before intake of glucose (Brown & Rother, 2009). Their analysis suggest that NAS have increased GLP-I secretion which reduce absorption time of sugar and hence insulin secretion is effected in antagonistic manner hence appetite and glucose variation can takes place (Brown et al., 2010). A study suggest that use of Soda/carbonated drink that are main source of artificial sweetener in children which induce overeating and results in weight gain in those children (Hill, 1965). Urine analysis shows that NCAS are excreted through body without being metabolized hence are non-caloric (Nabors, 2012).

Health benefits of NCAS

Prevention of dental decay

Dental decay or dental caries is problem that takes place by the excess use of carbohydrates as carbohydrates act as substrate for acid releasing bacteria, this acid than results in dental decay. This dental decay process is done when natural sugars are used while non-nutritive sugars don't act as substrate hence acid production decrease (Charu *et al.*, 2012).

Maintenance of weight

Short term studies indicate that aspartame helps in maintenance of body weight as it doesn't increase craving after using NCAS.

NCAS and diabetic patients

In a short term study conducted in 2002 it was observed that aspartame have no role in increase in blood glucose level hence it is beneficial for diabetic patients (Butchko *et al.*, 2002).

ASPARTAME

Aspartame is sallow fragrance-free powder composed of aspartic acid and phenylalanine and methanol

having molecular mass of C₁₄H₁₈N₂O. It was first approved by FDA in 1981 for its use in dry foods, in 1983 its use was prosper to carbonated drinks and in 1996 it was approved for general uses now its use is increased up to 4500 tons only in diet soda annually (Stonehouse et al., 2013). As it is a dipeptide, its solubility is least in water and alcohol while more in fats and oils. Diets that contain aspartame are breath mint, cereals, chewing gums, ice creams, sugar free gelatin, jams, jellies and other soft drinks (Meister, 2006).

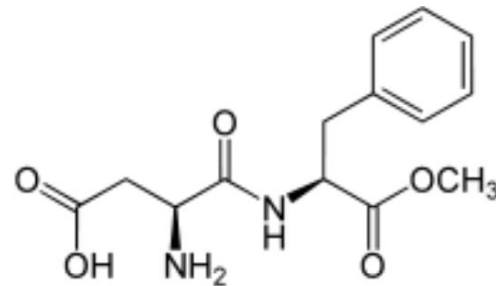


Fig. 1. Chemical structure of aspartame (Walters, 23 Sept. 2009).

Metabolism and health issues of Aspartame

Aspartame is composed of phenylalanine and aspartic acid; both are essential amino acids for human. Its breakdown takes place in intestinal lumen and mucosal cells and release of all three components in blood takes place. Within one hour of aspartame use concentration of phenylalanine doubled while of aspartate remain normal. Methanol converted into formaldehyde and formic acid results in metabolic acidosis (Palese & Tephly, 1975; O'Donnell, 2005). Some studies indicates that metabolism of aspartame takes place inside gut i.e. gut has esterases as well as proteases and further hydrolysis takes place inside mucosal or luminal cells (Wade, 2006). Soffritti and coworkers in 2007 established positive link between aspartame intake and different carcinomas and malignant tumors in both rat and human i.e. leukemia in both male and female, mammary glands cancers in females and various lymphomas including NHL and B-lymphocytic lymphoma etc. carcinogenic activity of aspartame increase if its intake is greater during gestation period. It is also observed that excess use of aspartame can leads to migraine, decrease in platelets, enlargement of spleen and liver (Whitehouse et al., 2008).

ACESULFAME POTASSIUM (ACE-K)

Chemically Ace-K is crystalline powder made of 5,6-dimethyl-1,2,3-oxathiazin-4(3H)-one-2,2-dioxide, first discovered in 1967 by Clauss and Jensen. The sweetness of is dependent upon its ring structure any modification in its structure results in decrease in sweetness. It is derived from β -diketones, β -oxocarbonic acid and alkynes that have ability react with halogen, sulfonyl and isocyanides, these substance cyclize in the presence of potassium hydroxide. Acesulfame is highly acidic compound the neutrality is achieved by potassium hydroxide (Arpe, 1978). Sweetness of Ace-K is 200 times greater than that of sucrose, but least sweet than that of saccharin and cyclamate and can be preserved for six year or more even at room temperature. The time of sweetness is slower and doesn't continue for longer time, but at high concentration it may taste bitter(Nabors, 2012).It is abundantly used in baked food as well as in soda bottles due to its stability in heat, aqueous environment and long half-life (Walters, 2009; Nabors, 2012).

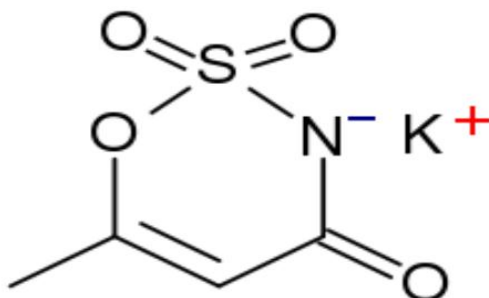


Fig. 2. Chemical structure of Ace-K (Nabors, 2012; Shallenberger, 1975)

Health study on Ace-K

Longitudinal study of Ace-K on animal and mice revealed that there is no role of Ace-K on cancer development and mutation in DNA if the daily intake is 0-9 mg/Kg. If the intake increases from that value it will result in increase in body weight and may be act as DNA damaging agent. Ace-K can be used to treat neurodegenerative disorders because its excess use results in decrease in neuro-protective activity of cell by inhibiting ATP production(Chadwick W, 2010). Evidence suggests that consumption of Ace-K induce increase in plasma insulin level. Acute symptoms related to Ace-K are headache, dizziness, nausea and vomiting, while long term exposure leads to leukemia as well as lymphomas (Whitehouse et al., 2008).

NEOTAME (N-(N-(3,3-DIMETHYLBUTYL)-L-ASPARTYL)-L-PHENYLALANINE 1-METHYL ESTER)

Neotame is derivative of phenylalanine and aspartic acid having sweetening influence 7000-8000 times greater than that of table sugar. As it is structurally resembled with aspartame its metabolism is same as that of aspartame, but it is highly stable in powdered form(Panchal et al., 2014; Nabors, 2012). Neotame is made powder by de-esterification at low temperature and are high functional stability

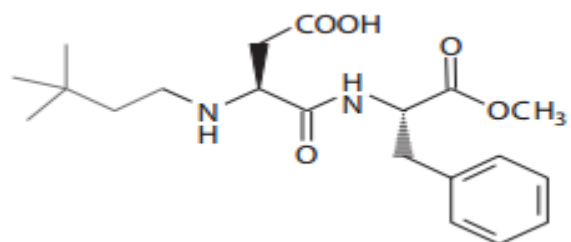


Fig. 3. Structure of Neotame (Nabors, 2012)

under range of temperature and pH. Neotame doesn't undergo intra-molecular cyclization so no DKP formed after its degradation (Nabors, 2012). Neotame is 30-60 time sweeter than aspartame due to modification in aspartate residue by tertiary butyl group. This modification helps in de-esterification resulting in easy clearance from plasma within 72 hours (EFSA, 2007). It's long term exposure may leads to low birth rate and excessive weight loss (Whitehouse et al., 2008).

Health benefits and issues

Short term study on health effects of Neotame suggests that there is no effect of Neotame dose during pregnancy and fetus development, and genetic variation. The only issue with this sweetener is that there is no labeling on this for those who are suffering from phenylketonuria(Nabors, 2012). It also induce increase in weight and food consumption (Mayhew et al., 2003).

SACCHARIN (O-SULFABENZAMIDE; 2,3-DIHYDRO-3-OXOBENZISOSULFONAZOLE)

Saccharin is available in three forms depending upon physical properties as well as ion added on it i.e. sodium, calcium and acid saccharin. It is white crystalline powder. Sodium saccharin has high solubility in water and used in food industry as flavoring agent., acid saccharin is slightly soluble in water and has its use in tobacco and toothpaste industries, calcium saccharin is also soluble in water

Table 1. Potential toxicity of artificial Sweeteners Whitehouse et al., 2008)

Common Name	Known Metabolites	ADI (mg/kg/d)	Manifestations of Toxicity in Humans	
			Acute	Chronic
Acesulfame-K		15	Headache	Clastogenic, genotoxic at high doses, thyroid tumors in rats
Aspartame	Methanol, aspartic acid, phenylalanine	50	Headache, dry mouth, dizziness, mood change, nausea, vomiting, reduced seizure threshold, thrombocytopenia	Lymphomas, leukemias in rats
Cyclamate	Cyclohexylamine	1		Bladder cancer in mice, testicular atrophy in mice
Neotame	De-esterified neotame, methanol	2	Headache, hepatotoxic at high doses	Lower birth rate, weight loss (due to decreased consumption at higher doses)
Saccharin	O-sulfamoylbenzoic acid	5	Nausea, vomiting, diarrhea	Cancer in offspring of breast-fed animals, low birth weight, bladder cancer, hepatotoxicity
Sucralose		5	Diarrhea	Thymus shrinkage and cecal enlargements in rats

ADI = acceptable daily intake.

and used in food industry (WHO, 2008). In 1921 it was investigated by Paul that it is 300 times sweeter than ordinary sugar (sucrose) and its sweetness is greater in aqueous solution reported by Dubois in 1991 (Paul, 1921). It is most widely used in beverages, food, cosmetics and pharmaceutical industries, as animal feed sweetener and electroplating of nickel (Salant, 1972).

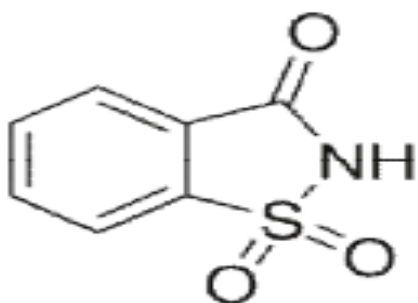


Fig. 4. Structure of Saccharin. Sodium and Calcium ions are substituted with SO residue. (PanchalIshan I, 2014)

Health issues

Longitudinal studies on its health effects suggest that it is source of bladder cancers in male if consumed daily. The acute problems regarding to saccharin include diarrhea and vomiting while long term exposure leads to cancer in offspring through breast feeding, low birth rate, bladder cancers and liver toxicity (Whitehouse et al., 2008).

CONCLUSION

Artificial sweeteners are low cost and non-nutritive sweetener having sweetness greater than that of normal table sugar. NCS are commonly used in baking industry, pharmaceutical industry and other food

industries. In spite of their beneficial effects these sweeteners have health issues as their long term exposure cause cancer and short term exposure cause headache, nausea and diarrhea. Recent epidemiological, clinical and laboratory findings question whether recommendations for the use of artificial sweeteners are indeed appropriate. A careful review of this literature by health professionals including physicians, epidemiologists, and dietitians is necessary to help consumers make well-informed decisions about their health. In this review, we have examined the existing evidence supporting a link between artificial sweetener use and their health risks. From all of this study it is concluded that a person should use sweeteners according to proper ADI in order to avoid problems.

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Moisture desorption isotherms characteristics of cocoa beans

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ABSTRACT

The present paper accounts for the study of the thermodynamic properties of moisture desorption characteristics of cocoa beans. Desorption isotherms of cocoa beans were determined experimentally using a static gravimetric method at 30, 45 and 60°C, providing water activity range of 0.10 – 0.90. Equilibrium moisture content data were correlated by GAB model, which presented good fit to the data, according to statistical procedures. The thermodynamic properties of cocoa beans as net isosteric heat, differential entropy, net integral enthalpy and net integral entropy were evaluated to provide an understanding of the properties of water and energy requirements associated with the sorption behaviour. Application of the enthalpy-entropy compensation theory to the desorption data of cocoa beans revealed that the process was enthalpy driven.

Keywords: Cocoa beans; Desorption; GAB model; Thermodynamic properties.

INTRODUCTION

Cocoa beans are the seeds of a tropical tree botanically known as *Theobroma cacao* (Family *Sterculiaceae*). They are usually processed through fermentation and drying upon harvesting during primary processing. Fermentation is usually carried out for 5-7 days in order to develop the flavour precursor and drying is carried out thereafter to terminate the fermentation process and to complete the browning reaction (Hii, 2004). The different processing procedures affect post-harvest techniques, in which to correctly conduct drying and storage operations. So, it is necessary to know the relationship between air temperature and relative humidity, and desirable conditions for preserving the product.

Sorption isotherms describe the relationship between the equilibrium moisture content and the water activity at constant temperatures. They give information about the water sorption mechanism and interactions between food components and water. The knowledge and understanding of sorption isotherms is necessary for the design and optimization of drying equipment, packages design, estimation of shelf-life stability and for calculating moisture changes that may occur during storage (Gabas *et al.*, 2007; Hassini *et al.*, 2015). Several mathematical models are cited in literature to describe the sorption behaviour of foodstuffs. However, the Guggenheim-Anderson-de Boer (GAB) model was recommended by the European Project Group Cost 90 (Wolf *et al.*, 1985) as a fundamental model to characterize water sorption in

foodstuff. The advantages of this model, when compared to the others, are its theoretical background and the physical meaning of its parameters (Oliveira *et al.*, 2011).

To further predict the physical properties and storage stability of cocoa beans, the thermodynamic properties of moisture sorption should be investigated. Thermodynamic functions include net isosteric heat, differential entropy, net integral enthalpy and entropy have drawn interest because it provides a more through interpretation of the sorption isotherm phenomenon and helps to understand better the sorption mechanism (Pérez-Alonzo *et al.*, 2006). The net isosteric heat of sorption can be used to estimate the energy requirements of drying and provides important information on the state of water in foodstuffs. The differential entropy of sorption is proportional to the number of available sorption sites at a specific energy level (Rosa *et al.*, 2010). Net integral enthalpy and entropy are used to explain the modes of moisture sorption by foodstuffs.

Thermodynamic data for cocoa beans in different processing procedures are scarce in the literature and is required to develop and design post-harvest equipment. It is in this context, that the present paper lies to determine the experimental desorption isotherms of cocoa beans at 30, 45 and 60 °C and to model the desorption isotherms of cocoa beans by using GAB model. The thermodynamic functions of desorption isotherms of cocoa beans are investigated. The theory of enthalpy-entropy compensation is applied.

MATERIALS AND METHODS

Experimental procedure

Desorption isotherms of cocoa beans were determined using the static method, which is based on using saturated salt solutions to maintain constant water activity of samples once equilibrium between room conditions and food sample is reached. Salt solutions used to obtain constant relative humidity were LiCl, KCH₃CO₂, MgCl₂, K₂CO₃, Mg(NO₃)₂, NaBr, SrCl₂, NaCl, KCl and BaCl₂. This group of salts allows obtaining a wide range of relative humidity from 11% to 90% as reported by Kiranoudis *et al.* (1993), Prette *et al.* (2013) and Koua *et al.* (2014). The static gravimetric is a method where diffusion is the only way of mass transfer between the product and the surrounding air. The air relative humidity is fixed by contact with saturated salts solution whose water vapour pressure at a given temperature is perfectly known. This method is commonly used for product sorption isotherms determination (Yazdani *et al.*, 2006; Brett *et al.*, 2009; Singh *et al.*, 2011; Koua *et al.*, 2014).

The samples were kept in triplicate in desiccators containing saturated salt solutions and placed in a temperature controlled cabinet, with an accuracy of ± 1°C at the selected temperatures of 30°C, 45°C and 60°C. The mass of each sample was measured with a first interval of 5 days and thereafter every 1 day. The duration for removal, weighing and replacing the samples was less than 10 s. This minimized the atmospheric moisture sorption during weighing. The thermodynamic equilibrium was considered to have been attained when the mass of the sample remained constant after three consecutive measurements at intervals of 24 h. The cocoa beans used in this study were purchased from the local retail market. Fresh cocoa beans were retrieved from the fruits and were fermented inside a wooden box for five days. The beans were manually turned every 48 h to ensure uniformity during fermentation. The initial moisture content was determined by the oven-drying method for 24 h at 105 °C and was found to be 1.10 kg water/kg db.

Data analysis and thermodynamic properties

Sorption isotherm

Experimental data of equilibrium moisture content (X_{eq}) of cocoa beans were fitted to GAB model (Eq. (1)). This model has three parameters that have physical meanings and the model has been found to adequately represent the water activity experimental

data over the rang of practical interest for most foods (Vigano *et al.*, 2012).

$$X_{eq} = \frac{X_m G K a_w}{(1 - K a_w)(1 + (G - 1) K a_w)}$$

Where X_m is the moisture content corresponding to the formation of a monomolecular layer on the internal surface; G is a constant related to the heat of sorption of the first layer on primary sites and K is a factor correcting properties of the multi-layer molecules with respect to the bulk liquid. The influence of temperature on GAB parameters G and K can be expressed with Arrhenius type equations (Goneli *et al.*, 2013):

$$G = G_0 \exp\left(\frac{\Delta H_G}{RT}\right)$$

$$K = K_0 \exp\left(\frac{\Delta H_K}{RT}\right)$$

where G_0 and K_0 are the pre-exponential factors, ΔH_G and ΔH_K are the enthalpies from the GAB model parameters, R is the universal gas constant ($R = 8.314 \text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$) and T is the absolute temperature.

The GAB parameters were estimated by fitting the mathematical model to the experimental data, using a non-linear regression analysis. The adequacy of the model was analysed based on the values of correlation coefficient (r^2), the mean relative percentage deviation modulus (E) in %, the root mean square error (RMSE) and residual plots (Basu *et al.*, 2006):

$$E = \frac{100}{N} \sum_{i=1}^N \frac{|X_{eq,exp} - X_{eq,pre}|}{X_{eq,exp}}$$

$$RMSE = \left[\frac{1}{N} \sum_{i=1}^N (X_{eq,exp} - X_{eq,pre})^2 \right]^{1/2}$$

where N is the number of observations; $X_{eq,exp}$ and $X_{eq,pre}$ are the experimental and predicted values of the equilibrium moisture content, respectively.

Treatments of sorption data according to the GAB model allow the evaluation of monolayer moisture content values of foodstuffs. The prediction of X_m values is important since deterioration of foodstuffs is very small below X_m . This is because water is strongly bound to the foodstuff below X_m and is not involved in any deteriorative reaction either as solvent or as one of the substrates (Ayrançi and Duman, 2005). Once the monolayer moisture content was known, the water surface area of product can be determined by Eq. (6)

(Rosa et al., 2010). The specific surface area plays an important role in determining the water binding properties of particulate products (Arslan and Togrul, 2005).

$$S_a = X_m \frac{1}{M_{H_2O}} A_{H_2O} N_A = 3.53 \times 10^3 X_m$$

where S_a is the product surface area ($m^2 \cdot g^{-1}$), M_{H_2O} is the molecular weight of water ($g \cdot mol^{-1}$), N_A is Avogadro's number (6×10^{23} molecules/mol) and A_{H_2O} is the area of one water molecule ($1.06 \times 10^{-19} m^2$).

Net isosteric heat of desorption

The net isosteric heat of desorption is defined as the isosteric heat of sorption minus the latent heat of the pure water vaporisation. It can be determined which is derived from the Clausius-Clapeyron equation (Singh et al., 2011), to data obtained from the GAB model:

$$\left[\frac{d \ln(a_w)}{d\left(\frac{1}{T}\right)} \right]_{X_{eq}} = -\frac{Q_{st} - L_v}{R} = -\frac{q_{st}}{R}$$

where q_{st} is the net isosteric heat of desorption ($kJ \cdot mol^{-1}$); Q_{st} is the isosteric heat of desorption ($kJ \cdot mol^{-1}$); L_v is the latent heat of the pure water vaporisation ($kJ \cdot mol^{-1}$).

Differential entropy

The differential entropy is related to the number of available sorption sites at a specific energy level (Madamba et al., 1996). Entropy defines the degree of order or randomness existing in the water-sorbent system which can help interpreting processes such as dissolution, crystallization and swelling (Hassini et al., 2015). The change in differential entropy (ΔS) of sorption was calculated from the Gibbs-Helmholtz equation (McMinn and Magee, 2003):

$$\Delta S = \frac{q_{st} - \Delta G}{T}$$

where ΔG is the Gibbs free energy and is calculated as:

$$\Delta G = -RT \ln(a_w)$$

Inserting Eq. (8) into Eq. (9), we can obtain Eq. (10)

$$\ln(a_w) = -\frac{q_{st}}{RT} + \frac{\Delta S}{R}$$

The net isosteric heat of sorption and differential entropy can be calculated from Eq. (10) by plotting $\ln(a_w)$ versus $1/T$, for given value of X_{eq} and then determining the slope ($-q_{st}/R$) and intercept ($\Delta S/R$).

Enthalpy-entropy compensation theory

The enthalpy-entropy compensation theory or isokinetic theory has been widely studied for several physical and chemical processes (Aguerre et al., 1986). The linear relationship between q_{st} and ΔS is given as (McMinn and Magee, 2003):

$$q_{st} = T_\beta \Delta S + \Delta G_\beta$$

The isokinetic temperature, T_β , has an important physical meaning as it represents the temperature at which all reactions in the series proceed at the same rate. The free energy, ΔG_β , at temperature T_β provides a criterion to estimate if the water sorption process is spontaneous ($-\Delta G_\beta$) or no ($+\Delta G_\beta$). Since there is a high degree of linear correlation between enthalpy and entropy, the compensation theory was assumed to be valid for sorption (Vishwakarma et al., 2011). To corroborate the compensation theory, a statistical analysis test was proposed (Krug et al. 1976). This involves a comparison of the isokinetic temperature (T_β) with the harmonic mean temperature (T_{hm}). The compensation theory only applies if $T_\beta \neq T_{hm}$. If $T_\beta > T_{hm}$, the sorption process is enthalpy driven, otherwise, the process is considered to be entropy controlled.

$$T_{hm} = \frac{n}{\sum_{i=1}^n \left(\frac{1}{T_i}\right)}$$

Spreading pressure

The spreading pressure or surface potential can be viewed as the force in the plane of the surface that must be exerted perpendicular to each unit length of edge to keep the surface from spreading (Smith et al., 2001). The spreading pressure which is the surface excess free energy is calculated using an analytical procedure described by Iglesias et al. (1976):

$$\pi = \frac{K_B T}{A_{H_2O}} \int_0^{a_w} \frac{\theta}{a_w} da_w$$

where the moisture ratio θ is:

$$\theta = \frac{X_{eq}}{X_m}$$

K_B is the Boltzmann's constant ($K_B = 1.38 \times 10^{-23} \text{ JK}^{-1}$) and π is the spreading pressure (J.m^{-2}).

The value of $a_w = 0$ will make the integral of Eq. (13) to be indeterminate. Therefore, the spreading pressure was calculated by integrating the GAB model into Eq. (13). The GAB model presents an empirical relation between a_w and X_{eq} :

$$\frac{a_w}{X_{eq}} = \frac{K}{X_m} \left(\frac{1-G}{G} \right) a_w^2 + \frac{1}{X_m} \left(\frac{G-2}{G} \right) a_w + \frac{1}{X_m G K}$$

Substituting Eq. (15) into Eq. (13) and integrating gives the spreading pressure at different temperatures on the basis of surface area per sorption site or area per one molecule of water on each sorption site as:

$$\pi = \frac{K_B T}{A_{H_2O}} \ln \left[\frac{1 + K a_w (G-1)}{1 - K a_w} \right]$$

Net integral enthalpy and entropy

The net integral enthalpy or net equilibrium heat of sorption is regarded as the difference in surface tension between bare sorption sites in the solid and sites with adsorbed molecules (Al-Muhtaseb *et al.*, 2004). It provides an indication of the binding strength of water molecules to the solid, and has some bearings on the energy balance of drying and freezing operations. The net integral enthalpy (q_{in}) was determined in a similar manner as the isosteric heat but at a constant spreading pressure (Togrul and Arslan, 2007).

$$\left[\frac{d \ln(a_w)}{d \left(\frac{1}{T} \right)} \right]_{\pi} = - \frac{q_{in}}{R}$$

A plot of $\ln(a_w)$ versus $1/T$ at constant spreading pressure gives the net integral enthalpy from the slope. Net integral enthalpy was used to determine the net integral entropy associated with the sorption process. The net integral entropy describes the degree of disorder and randomness of motion of water molecules. It quantifies the mobility of the adsorbed water molecules, and indicates the degree to which the water-substrate interaction exceeds that of the water molecules. The net integral entropy (ΔS_{in}) is given by (Togrul and Arslan, 2006):

$$\Delta S_{in} = - \frac{q_{in}}{T} - R \ln(a_w^*)$$

where a_w^* is the geometric mean water activity obtained at constant spreading pressure at different temperatures.

RESULTS AND DISCUSSIONS

Desorption isotherm

The experimental desorption isotherm data for cocoa beans at 30, 45 and 60 °C are shown in Fig. 1. Sigmoid-shaped type is observed for all temperatures studied. This shape is characteristic of amorphous products rich in hydrophilic compounds (Al-Muhtaseb *et al.*, 2004). This is typical of type II isotherms and has been reported for starchy products as oat flour and rice flour (Brett *et al.*, 2009), tea (Chen and Weng, 2010), cocoa beans (Oliveira *et al.*, 2011), guar grains and splits (Vishwakarma *et al.*, 2011). The equilibrium moisture content decreases with the temperature at constant water activity. It could be explained that the temperature affected the mobility of water molecules and dynamical equilibrium between water vapour and adsorbed phase (Rhim and Lee, 2009). Equilibrium moisture content increases also with increase in water activity for all temperatures. This behaviour may be attributed to the excitation states of molecules. At increased temperatures, molecules are in an increased state of excitation, thus increasing their distance decreases the attractive forces between them (Kaya and Aydin, 2009). This leads to a decrease in the degree of water sorption at a given water activity with increasing temperature (Jamali *et al.*, 2006). Whereas Mazza and Lemaguer (1980) suggested that increase in temperature induces physical and chemical changes in the product that can reduce the number of active sites for water binding. The temperature dependence of the equilibrium moisture content has an important practical bearing on chemical and microbiological reactions associated with spoilage (Al-Muhtaseb *et al.*, 2004). At the same moisture content, higher temperatures entail a higher water activity and consequently faster rates of deterioration (Van den Berg and Bruin, 1981).

Estimated parameters of GAB model and statistical parameters used in order to analyse model adequacy are presented in Table 1. GAB model presented random residual plots of equilibrium moisture content experimental data of cocoa beans at all temperatures. Also, low values of E and RMSE were encountered along with high values of r^2 . These results indicate that

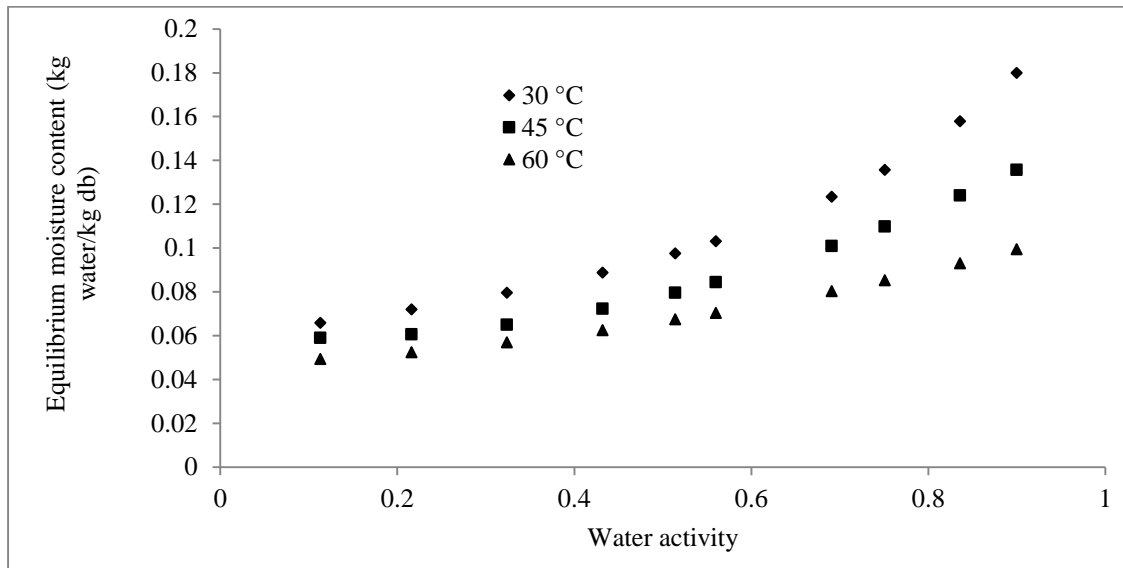


Fig. 1. Experimental desorption isotherm of cocoa beans at 30, 45 and 60 °C with water activity

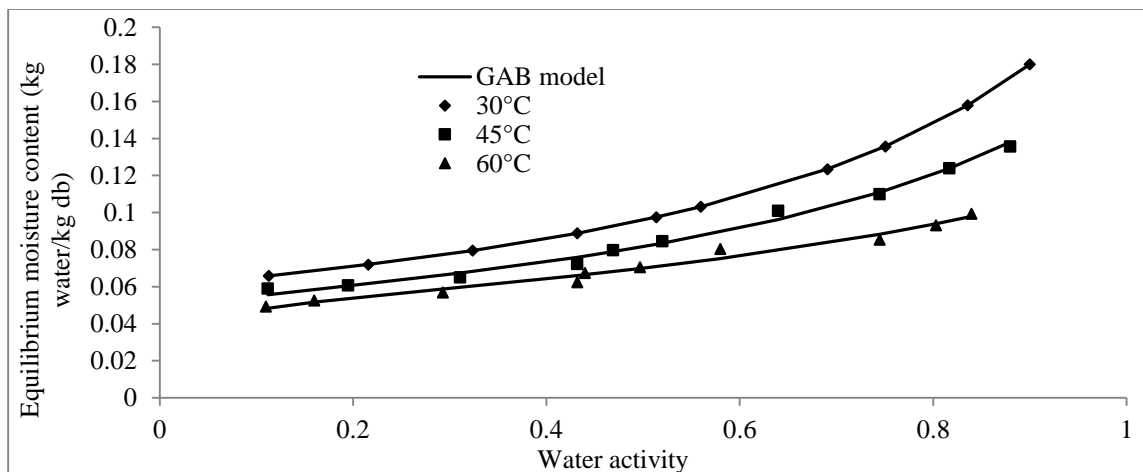


Fig. 2. Experimental desorption isotherms of cocoa beans at 30, 45 and 60 °C and estimated curves with GAB model

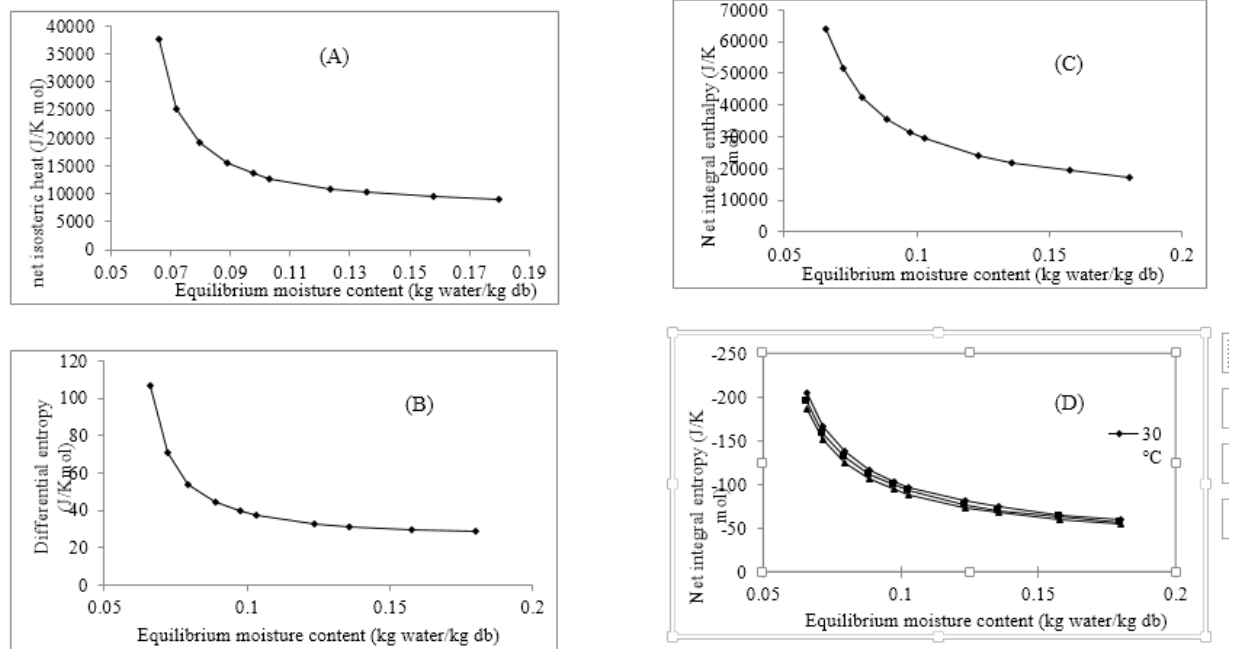


Fig. 3. Net isosteric heat (A), Differential entropy (B), Net integral enthalpy (C) and Net integral entropy of desorption of cocoa beans at 30 °C, 45 °C and 60 °C with equilibrium moistent content

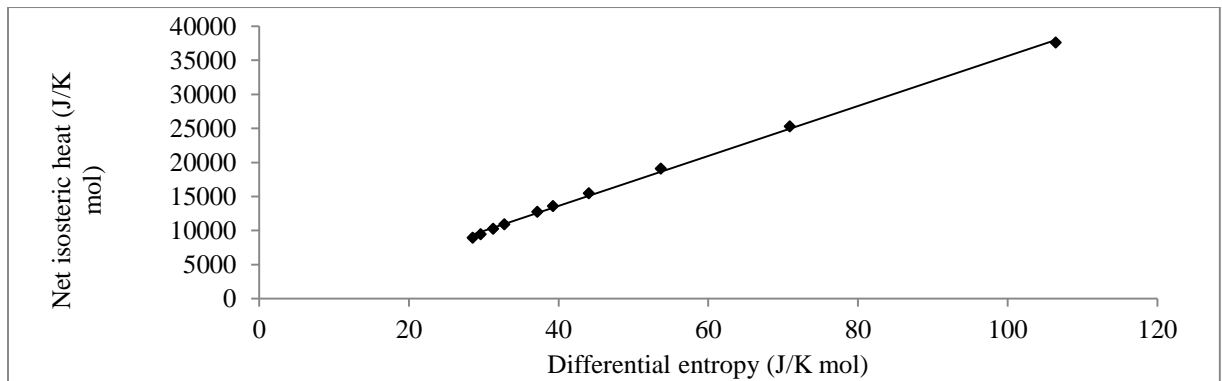


Fig.4. Net isosteric heat versus differential entropy relationship for desorption in cocoa beans

Table 1.Parameter estimated values, mean relative percentage deviation modulus (E), root mean square error (RMSE), correlation coefficient (r^2) and residual plots for GAB model to cocoa beans desorption process

Temperature (°C)	Parameters			r^2	E (%)	RMSE	Residual plots
	Xm	G	K				
30	0.0606	3729.9748	0.7372	0.999	0.0392	0.00006	Random
45	0.0535	279.5272	0.6970	0.990	2.4083	0.0025	Random
60	0.0508	115.6945	0.5789	0.978	2.7397	0.0024	Random

GAB model is adequate to estimate the equilibrium moisture content of cocoa beans (Fig. 2).

Monolayer moisture content of cocoa beans obtained using GAB model was found to be 0.061, 0.054 and 0.051 kg water/ kg dry basis for 30, 45 and 60 °C, respectively. The decrease in monolayer moisture contents with increase in temperature, as observed in this study, may be due to a reduction in the total number of active sites for water binding as a result of physical and/or chemical changes in the product induced by temperature (Iglesias and Chirife, 1976). Also, according to Palipane and Driscoll (1992), it could be that with increase in temperature, the water molecules got activated due to an increase in their energy level, causing them to become less stable and to break away from the water binding sites of the food, thus decreasing the monolayer moisture content. These observations imply that the anticipated temperature of storage of the cocoa beans would determine the optimum moisture content for maximum self-stability. Table 2 gives the results of the regression analysis of GAB model parameters. The positive value for ΔH_G in desorption mode indicates that the sorption heat of water monolayer molecules is higher than the sorption heat of water multilayer molecules. The positive value for ΔH_k in desorption mode indicates that the condensation heat of water vapour is higher than the sorption heat of water multilayer molecules.

Table 2. Estimated values of GAB model constants and its respective enthalpy values

Constants		Enthalpy (KJ/K mol)	
G ₀	K ₀	ΔH_G	ΔH_K
3.96x10 ⁻¹⁴	0.0528	97.89	6.70

The values of the water surface area of cocoa beans determined from Eq. (6) using the monolayer moisture contents values obtained from the GAB model were 213.92, 188.86 and 179.32 m²/g for 30, 45 and 60 °C, respectively. These results indicate that the availability of surface areas for hydrophilic binding decrease with increasing temperature. The calculated surface area values of cocoa beans were within the range commonly obtained for foodstuffs (100 – 250 m².g⁻¹) (Cassini *et al.*, 2006). Calzetta *et al.* (2000) indicated that the large surface area of many biopolymers is due to the existence of an intrinsic microporous structure in the foodstuffs.

Thermodynamic properties

Net isosteric heat of desorption

The net isosteric heat of desorption for cocoa beans versus equilibrium moisture content obtained by applying Clausius-Clapeyron equation is shown in Fig. 3(A). As seen in Fig. 3(A), the net isosteric heat of desorption decreases with increasing equilibrium moisture content, from 37.39 to 9.01 kJ/mol with a variation in the equilibrium moisture content ranging from 0.066 to 0.180 kg water/kg dry basis. This decrease can be qualitatively explained by considering that sorption initially occurs at the most active sites, giving rise to the greatest interaction energy. As the equilibrium moisture content increases, the sites available for water sorption decrease, resulting in lower values of net isosteric heat of desorption (Tsami *et al.*, 1990; Polatoglu *et al.*, 2011). Similar results have been reported for various biological products in literature (Rosa *et al.*, 2010; Oliveira *et al.*, 2011; Goneli *et al.*, 2013; Koua *et al.* 2014; Ouertani *et al.*, 2014; Yang *et al.*, 2015; Hassini *et al.*, 2015). The net isosteric heat versus equilibrium moisture content results is adequately represented by a power law relation of the form:

$$q_{st} = \exp(0.929X_{eq}^{-0.46}) \quad \text{with } r^2 = 0.907$$

Differential entropy

The differential entropy decreases with increasing equilibrium moisture content, tending to become constant at high values of equilibrium moisture content as shown in Fig. 3(B). It ranged from 106.42 to 28.53 J/molK with equilibrium moisture content ranging from 0.066 to 0.180 kg water/kg dry basis. At low water activity range, a decrease in differential entropy values is possibly caused by the strongest binding sites with water molecules and the solid as suggested by McMinn and Magee (2003). The differential desorption entropy data displays a strong dependence on equilibrium moisture content as stated by Kaya and Kahyaoglu (2007). The net differential entropy versus equilibrium moisture content results is adequately represented by a power law relation of the form:

$$\Delta S = \exp(2.290X_{eq}^{-0.19}) \quad \text{with } r^2 = 0.920$$

Enthalpy-entropy compensation theory

A good straight line ($r^2 = 0.998$) related the net desorption isosteric heat to the differential desorption entropy for the cocoa beans, at different temperatures (Fig. 4). This behaviour confirms the enthalpy-entropy compensation hypothesis (Leffer and Grunwald, 1963). The parameters T_β and ΔG_β (Eq. (11)) were calculated from the experimental data by linear regression as 367K and -1051 kJ/kg, respectively. Confirmation of the application of the compensation theory is provided by calculation of an harmonic mean temperature (T_{hm}) value of 317.68 K. As $T_\beta \neq T_{hm}$, the compensation theory can also be applied and the water desorption in cocoa beans is spontaneous ($\Delta G_\beta < 0$). As $T_\beta > T_{hm}$, the sorption process is enthalpy driven. Similar results have been obtained by Goneli *et al.* (2013), Thanuja and Ravindra (2014).

Spreading pressure

The spreading pressure values were evaluated using the analytical expression (Eq. (16)). The spreading pressure in desorption process of cocoa beans are represented in Table 3. The results shown that the spreading pressure increased with increasing water activity and, at a given water activity, decreased with increasing temperature. The trends of spreading pressures with respect to temperature and water activity for desorption were similar to those determined by McMinn and Magee (2003) for potato, Thanuja and Ravindra (2014) for cheese-puri mix.

Table 3. Spreading pressures for cocoa beans at different temperatures

Water activity	Spreading pressure (J/m ²)		
	30 °C	45 °C	60 °C
0.113	0.2300	0.1328	0.0946
0.216	0.2589	0.1861	0.1106
0.324	0.2789	0.2093	0.1389
0.432	0.2946	0.2276	0.1595
0.514	0.3051	0.2325	0.1606
0.56	0.3107	0.2390	0.1677
0.691	0.3261	0.2534	0.1772
0.751	0.3330	0.2656	0.1946
0.836	0.3433	0.2740	0.2004

Net integral enthalpy and entropy

The net integral enthalpy of desorption decreased with increasing equilibrium moisture content as shown in Fig. 3(C). This behaviour can be attributed to the

monolayer covering of the products (Thanuja and Ravindra, 2014). At low equilibrium moisture content, water is adsorbed on the most accessible locations on the exterior surface of the solid. At higher equilibrium moisture content, the decreasing in net integral enthalpy could be attributed to the covering of favourable locations and formation of multiple layers of sorbed water. The net integral enthalpy versus equilibrium moisture content results is adequately represented by a power law relation of the form:

$$q_{in} = \exp(7.868X_{eq}^{-0.12}) \quad \text{with } r^2 = 0.974$$

The net integral entropy of desorption increased with increasing equilibrium moisture content (Fig. 3(D)). As cocoa beans adsorbed moisture, the entropy diminished to a minimum point that is considered as that of maximum stability, because it is where the water molecules achieve a more ordered arrangement within the solid. The minimum integral entropy value was found to - 204.59, - 194.62 and - 185.55 J/K mol at 30, 45 and 60 °C, respectively. This minimum value was expected to arise where strong bonding occurs between adsorbent and adsorbate which corresponds to less water being available for spoilage reactions. The minimum integral entropy values of moisture sorption in cocoa beans were observed at equilibrium moisture content of 0.065, 0.058 and 0.049 kg water/kg dry basis, respectively. This is consistent with the estimated monolayer moisture content values by the GAB model (Table 1). For all of the equilibrium moisture content range the net integral entropy values were negative. Negative net integral entropy values were attributed to the existence of chemical adsorption and/or structural modifications of the adsorbent (Iglesias *et al.*, 1976). The monolayer moisture content is the safest equilibrium moisture content for storage purposes, providing increased time period with minimum quality loss at a certain temperature during preservation. Values below monolayer moisture content slow down deteriorative reactions, pest attacks and respiration rate of the product.

The net integral entropy versus equilibrium moisture content results is adequately represented by a power law relation of the form:

$$\Delta S_{in} = -\exp(2.586X_{eq}^{-0.25}) \quad \text{with } r^2 = 0.979 \text{ at } 30^\circ \text{C}$$

$$\Delta S_{in} = -\exp(2.547X_{eq}^{-0.25}) \quad \text{with } r^2 = 0.979 \text{ at } 45^\circ \text{C}$$

$$\Delta S_{in} = -\exp(2.511X_{eq}^{-0.26}) \quad \text{with } r^2 = 0.979 \text{ at } 60^\circ \text{C}$$

CONCLUSION

The desorption isotherms of cocoa beans were determined at the temperatures of 30, 45 and 60 °C, using the static gravimetric method. Equilibrium moisture contents were found to decrease with increasing temperature at constant water activity. They were also found to increase with increasing water activity at a given temperature. According to the results, GAB model described well the desorption process of cocoa beans. Also, monolayer moisture content decreased with increased drying temperature. Thermodynamic parameters such as net isosteric heat, differential entropy, net integral enthalpy and entropy, were estimated for desorption process of cocoa beans. Evaluation of these parameters during drying process is of fundamental importance to the correct preservation of the product, as well providing parameters to design and develop the several equipments utilized on the main postharvest operations. All thermodynamic functions presented high correlation with the equilibrium moisture content of cocoa beans and the enthalpy-entropy compensation theory exists.

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Herbs and spices as a potential antimicrobial agents for food application

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ABSTRACT

Synthetic food additives pose serious threat to the consumers regarding food safety (which is a prime demand of the consumers). Herbs and spices as natural foodstuffs are the replacers synthetically formulated additives, leaving safety concern and providing prime quality food products. These naturally occurring additives have been traditionally used as medicine, flavoring agents and for the food preservation. Spices have been regarded as permanent ingredient in household culinary practices and also adopted as a part of daily diets worldwide. They are blessed with numerous functional benefits as their small quantity can enhance flavor and used as naturally antimicrobial agents. Current research unveiled different spices and herbs for their antimicrobial activities and functional effects besides their flavor and fragrance traits. Clove, garlic, cinnamon, rosemary, cumin, oregano, bay, thyme, mustard, basil, pepper, ginger and sage have been reported for their antimicrobial activity against spoilage and pathogenic bacteria along fungi *Staphylococcus* spp., *Escherichia* spp., *Listeria* spp., *Pseudomonas* spp., *Aspergillus* spp., *Cladosporium* spp. and *Salmonella* spp. Type of microorganism, nature of food product, type of spices essential oils and extracts determine the potential of antimicrobial activity.

Keywords: Plant extract, essential oil, spices, herbs, antimicrobial activity

INTRODUCTION

Spices are to be defined as any flavor imparting aromatic plant substance which is meant for the seasoning than nutrition. It adds savor or spiciness to the food products or drinks. Historically spices have been used preserving the foods products since decades. The essential oils having phytochemicals are good source of natural antimicrobial agents. Egyptians adopted herbs and spices in their daily food. These traditional items were ascended down to the Asian region including China, India and Pakistan. Different spices (clove, ginger, cinnamon, mint and garlic) are utilized as nutraceutical and for the remedy of different diseases (Kordali *et al.*, 2005).

In the ancient history about in 200 BC, Chinese used these spices to refresh their breath during their conversation with the emperor for their good sake and also to remove the off odor. The extracts and essential oils having secret constituents that acts to inhibit the microbial growth and prevent the deteriorative activities. Spices and herbs have also played role in the dramatic uphill of the civilization in the account of nations. The palatability of different dishes is enhanced by the spectacular aroma and tanginess of spices and herbs. Pharmacological and nutraceutical based industry is thoroughly relying on

the herbs and species for the preparation of numerous medicines. These are helpful to the cure of different disease as ginger is proven handful for the treatment of the cancer and different nervous system related diseases. Sensory attributes of the product (Flavor, color and taste) are improved and off-odors are masked by the species. Secret ingredients for these characters are the volatile oils and oleoresins (Proestos *et al.*, 2008).

Conventionally used spices include clove, cinnamon bark, dill seed, coriander seed, garlic, licorice root, ginger root, mint oil, paprika, onion, parsley herb/root, peppermint leaf and oil, thyme, rosemary, sage, white mustard seed and turmeric root. Dyspepsia and gastrointestinal disturbances are mainly cured by these herbs and spices (Silva *et al.*, 2007). Food pathogens are a big trouble for food handlers as they deteriorate and spoil the food. The antimicrobials have been incorporated in the food products due to following concerns: (1) to enhance food preservation by controlling food spoilage microorganisms (2) to hinder or suppress pathogenic microbes (food safety). There are different sources of naturally derived antimicrobials such as plants, animal and microbes. The effectiveness of the essential oils was reported by different scientists and

it was found that the oregano is the most effective essential oil against food pathogens. The least effective oil was recorded for cilantro against pathogens. These are regarded as natural antimicrobial agents used for the preservation of food products (Burt, 2004).

The shelf life of the semi-processed and processed food can be extended by reducing the viability of microbes. It's all due to the application of antimicrobial compounds present in spices and herbs. These antimicrobial properties are helpful not only to improve shelf life but to improve the taste of the product. Herbs and spices have inbuilt self-defense against the pathogenic and infectious microorganisms (Kim *et al.*, 2003). Fresh fruit juices and processed vegetables have plenty of space for the application of these health adjuncts. Different spices like ginger, clove, mint, garlic and cinnamon have been used for the remedies of different diseases in India (Kordali *et al.*, 2005). Moreover current researchers focus on its mechanism of action, toxicological effects and sensory attributes, but still plenty to come (Burt, 2004; Davidson, 2006; Gaysinsky and Weiss, 2007). The range of minimum inhibitory concentration of the extracts of herbs and spices lies between 0.2-10 μ ml⁻¹. These essential extracts are handful against different pathogens like *Staphylococcus aureus*, *L. monocytogenes*, *Shigella dysenteriae*, *Salmonella typhimurium*, *Escherichia coli* and *Bacillus subtilis* (Burt, 2004; Brandi *et al.*, 2006). Generally, because of the lipopolysaccharide outer membrane of Gram-negative bacteria are less sensitive to the antimicrobial agents, that restricts diffusion of the hydrophobic compounds. However, it does not mean that Gram-positive bacteria are always more vulnerable (Burt, 2004). Gram-negative bacteria are typically more unwilling to the plant-origin antimicrobial compounds and still it shows no effect as compared to Gram-positive bacteria (Stefanello *et al.*, 2008).

Some phytochemicals with stronger anti-microbial activity be effective at MIC 1000 ppm. It can vary depending upon different conditions (Ceylan and Fung, 2004). The proposed antimicrobial effect highlights that microbial cells are prone to the extracts. They also attack the cell membrane made up of phospholipids bilayer and denature the enzymes. The degradation of fatty acids results in the breakdown of unsaturated fatty acids. In contrast with the natural antimicrobials, the chemical antimicrobials are not safe because they are carcinogenic in nature. The point of concern for the use of chemical preservatives is the toxicity and safety of these chemicals. At the present time,

consumers are more concerned with the safety requirement of these chemicals (Skandamis *et al.*, 2001). Natural antimicrobial are good replacers as they suppress the growth of different microbes. It is also helpful to reduce the processing and preservation costs, preventing the contamination and reducing the necessity for antibiotics. While, health issues could be solve by strengthening immune system (Abou-taleb and Kawai, 2008; Fisher and Phillips, 2008).

SPICES

In food products spices are mostly used as natural antimicrobial agents. For thousands of years, it has been used for preserving the foods and as food additives to improve flavor and aroma. The antimicrobial activities of most of the spices and their active components have been studied. Investigations confirm that onion, garlic, cloves, cinnamon, black paper, ginger, and other spices reduce the growth of food borne pathogens, yeast, molds or spoilage bacteria. Different spices exhibits different antimicrobial and antibacterial property against different strains within the same species. Different spices have different antimicrobial/antimicrobial activity depending on the type of spices, nature of the spices (such as dried, fresh, or extracted forms) and also depend upon the harvesting seasons and geological sources. Hence, it is proved that spices essential oils are more powerfully antimicrobial than it is accounted as an additive (Gaysinsky and Weiss, 2007).

CLOVE

Clove belongs to *Eugenia caryophyllata* that is a tree (*Syzygium aromaticum*) and it is aromatic dried buds in nature. It is worn as a spice in almost all the world's fare. The word "Clove" is derived from the French word 'Clou' and the English word 'Clou', both have same meaning 'nail' - from the likeness of the flower bud of the Clove tree to a broad-headed nail (Li *et al.*, 2005). Cloves are inhabitant to the Moluccas, previously known as the Spice Islands of Indonesia. They have been consumed in Asia for more than 2,000 years. The countries, in which it is mostly cultivated, are Sri Lanka, Indonesia, China and Madagascar (Bureau of Drug Administration of China, 1989). Bud Oil of the Clove has certain properties include insecticidal, antioxidant, antibacterial and antifungal agent. By custom it has been used in the food as a flavoring agent and preservative as antimicrobial substance (Velluti *et al.*, 2003). The production of clove has averaged approximately 80,000 ton/year, in recent years. Indonesia is the world's largest producer at 50,000-60,000 ton/year. USA, Saudi Arabia, France and

India are the main importing countries of clove. Clove has vital role as a spice in trading and has high value for their therapeutic activity. Clove is a rich source of manganese, vitamin K, vitamin C, dietary fiber, omega 3 fatty acids and an excellent source of calcium and magnesium. Iron, proteins, carbohydrates, phosphorus, sodium, potassium, and hydrochloric acid is also present in clove (Kim *et al.*, 1998). The most important compounds of clove is phenylpropene eugenol and it is well known aromatic compound. Eugenol comprises 70-90% and remaining 15% dry weight (Shobana and Naidu, 2000). Microorganisms such as *Aspergillus* sp., *Alternaria* sp., *Lactobacillus* sp., *Cunninghamella* sp., *Fusarium* sp., *Mucor* sp., *Clostridium* sp., *Salmonella* sp. *Bacillus* sp. and *Penicillium* sp. can easily controlled by using clove essential oil (Soliman and Badaea, 2002). Since ancient times clove has been used as medicine. Eugenol is the main necessary component of clove oil that has significantly higher antimicrobial properties against food micro-organisms. By the application of clove essential oil molds, yeasts and bacterial growth could be controlled (Burt, 2004). Eugenol is the main bioactive component of clove, which make it effective against various health ailments e.g. digestive tract cancers and joint inflammation (Friedman *et al.*, 2002). The cloves are anti-ulcerogenic, anti-inflammatory, anti-mutagenic, anti-oxidant, antiparasitic and antithrombotic. Dried flower buds of cloves used for acne, warts, parasites and scars (Miyazawa and Hisama, 2003).

GARLIC

Garlic belongs to Alliaceae family and its botanical name is *Allium sativum*. It has hot, pungent flavor that mellows and sweetens with cooking. It has been used for culinary as well as for medicinal purpose. Garlic is used as medicine for many years. It has been also claimed that garlic prevent from cardiovascular diseases, high blood pressure and high cholesterol. It also protects against various cancer as well as improves immune system response. For six month daily dose of garlic in the form of extract 1ml/kg body weight can reduce the oxidative stress in the patient's blood (Karuppiah and Rajaram, 2012).

Garlic has antimicrobial activity and without showing cytotoxic effect on normal cells it has ability to induced apoptosis. It has great antimicrobial potential against gram positive bacteria, gram negative bacteria and food borne pathogens including *Salmonella*, *Streptococcus*, *Staphylococcus*, *Coliform*, *Lactobacillus* and some anaerobic bacteria (Purwanti *et al.*, 2014).

Allicin is considerable bioactive compound present in garlic which shows strong antimicrobial activity and scavenging antioxidant compound (Chung, 2006). The allinase enzyme accountable for thiosulfonate conversion and becomes inactivated below 3.5 pH or with heat. Microwave radiation will damage allinase enzyme activity within one minute. (Pedrazza-Cheverri *et al.*, 2006).

Garlic indicates anti-inflammatory activity and immunomodulatory effect. It can be used in treatment of arterial hypertension due to its hypolipemic and antioxidant properties (Kyo *et al.*, 2001). Among others, garlic possesses anti-atherosclerotic and anti-thrombotic activity (Duda *et al.*, 2008). Bioactive compounds derived from garlic demonstrate anticancer activities (Lai *et al.*, 2012) and they are potential to decrease the risk of cancer metastasis inhibiting tumor cell motility (Park *et al.*, 2011).

GINGER

The scientific name of ginger is *Zingiber officinale*. It belongs to small family, that has 800 species and 45 genera, Zingiberaceae. Ginger has active component that known as curcumin (Milner, 2006). It interferes in the formation of cell membrane of some pathogens like *Salmonella* and *E. coli* (Amagase, 2006). It is a plant growing in 1-3 feet in height. A club like spike of yellowish purple flower in cultivation (Tyler, 2002). Ginger is truly domestic remedy of various infections. Fresh ginger is used for cold diseases such as asthma, nausea, colic, cough, dyspepsia, swelling, and heart palpitation. A paste of powder dried ginger was used to relief headache when it is mixed with honey. It also use to alley nausea (Karuppiah and Rajaram, 2012). Mixture of honey and ginger show high antimicrobial activity against the Gram positive bacteria and Gram negative bacteria (Patel *et al.*, 2011).

CINNAMON

Cinnamon belongs to genus *Cinnamomum* and family Lauraceae. For thousands of year it is used for culinary as well as for medicinal perspective (Ranasinghe *et al.*, 2012). The production rate of this spice is 27500-35000 tons/year (Madan *et al.*, 2004). The important component identified is *C. Zeylanicum*. This component consists of two chemical volatile phenols and poly phenols. Cinnamon normally refers to the bark of *C. Zeylanicum* and *C. Aromaticum* that is used for the preparation of many beverages, chocolate, liquors and spicy candies. One of the most important properties of cinnamon is that it shows as antibacterial agent against the Gram positive bacteria

and Gram negative bacteria (Krishnamoorthy *et al.*, 2004). Major antibacterial component in cinnamon is cinnamaldehyde. Cinnamaldehyde reported to restrain the growth of *E.coli*, *S. aureus* and *Salmonella Typhimurium* (Hoque *et al.*, 2008). Furthermore, it also possesses strong antifungal, antibacterial, larvicidal, antitermitic, insecticidal and nematocidal properties (Kim *et al.*, 2006).

HERBS

The quality and quantity of food is mainly reduced by the presence of food borne and spoilage microorganisms. The disease causing microbes including fungi, bacteria, yeasts and molds produce mycotoxins, aflatoxins and various carcinogenic substances. Certain chemical additives and preservatives are being extensively used for preserving food. But these chemicals render the food toxic and carcinogenic. Increased concern of consumers regarding safe and healthy food creates an urge to use natural products for food preservation. Nature has provided plant essential oils with numerous antimicrobial and antibacterial properties. Natural herbs and spices contains antimicrobial effects which can be used for the preservation of food (Omidbeygi *et al.*, 2007).

The strongest antimicrobial properties are processed by the essential oils of thyme, oregano, mint, clove and cinnamon. These essential oils are highly effective in curing human diseases, wounds and infections. Essential oils from herbs acquire strong antibiotic activity and act as powerful tool against bacteria which are drug resistant. Essential oils that contains high amount of phenolic compounds are characterized to be the best antibacterial (Sienkiewicz *et al.*, 2012).

THYME

An evergreen herb, thyme commonly used in medicines, culinary and as ornamental plant. *Thymus citriodorus*, *Thymus herba-barona*, *Thymus pseudolanuginosus*, *Thymus serpyllum*, *Thymus praecox* and *Thymus vulgaris* are the important species and cultivars of Thymus plant. Essential oil of Thyme along with oregano is being used as preservatives in meat products and found to be very useful in controlling food spoilage microorganisms. These aromatic oils enhance flavor and act as antimicrobials. The seven main components of thymus essential oils are α -pinene, p-cymene, γ -terpinene, linalool, α -terpineol, thymol and carvacrol. The major mechanism of antimicrobial components thymol and carvacrol is to deteriorate the outermost membrane of gram negative bacteria. This releases liposaccharides and ATP becomes permeable to the

cytoplasmic membrane. This mechanism works best against *Escherichia coli* and *Salmonella* spp. (Boskovic *et al.*, 2015). It has been reported that thymol is effective to destroy coliforms, when used at the concentration of 250, 500 and 750 mg/kg of beef minced patties and stored for 16 days under ordinary or modified packaging at refrigeration temperature. According to another research, it has been found that by covering the surface of meat products by 0.8% thymol, 2-3 log units had been decreased (Emiroglu *et al.*, 2010).

OREGANO

Oregano (*Origanum vulgare*), a perennial herb, sometimes called wild marjoram belongs to mint family. The basic components which contribute properties to the herb are carvacrol, thymol, limonene, pinene, ocimene, and caryophyllene. monoterpenoids and monoterpenes are the chemical components of essential oil of oregano which contributes sensory and chemical attributes to the products. Oregano along with marjoram essential oil is effective to control gram positive and negative bacteria (Goni *et al.*, 2009). Oregano is a strong weapon against the two food spoilage molds *Aspergillus niger* and *Aspergillus flavus*. Oregano has the highest potential compared with other essential oils to restrict mycelium growth by 57% when used at 2mL concentration/ 18mL culture medium concentration. Many possible mode of action of these antimicrobial have been reported. It has been studied that these antimicrobials contains phenolic compounds which at low concentrations effects enzyme activity, denatures protein and loss of cell permeability (Viuda-Martos *et al.*, 2007) Silva and Junior (2010) analyzed essential oils from oregano for its antibacterial activity against multi resistant bacteria including *E. coli*, *P. aeruginosa*, *E. faecalis*, *K. pneumonia* and *Acinetobacter baumannii*. The growth of all the bacterial strains was inhibited at the concentrations of 0.125% except *P. aeruginosa* was inhibited at the concentration of 0.5%. Antibacterial property of oregano EO in meat products was investigated and found that bacteria which are gram positive are more vulnerable to them.

ROSEMARY

Rosemary (*Rosmarinus officinalis*), an evergreen, aromatic, perennial herb, belongs to genus *Rosmarinus*. It has needle like leaves and is grown as decorative plant in gardens. Rosemary leaves either fresh or dried are used in culinary to give aroma to roast and barbequed meat particularly. Rosemary EO is being used in pharmaceutical applications, as taste enhancer, antioxidant, antimicrobial and food

preservative. Rosemary contains antibacterial and antifungal agents against bacteria and fungi. Rosemary contains carnosic acid and carnosol as antioxidants which are mainly responsible for high antimicrobial activity. Rosemary leaves extract contains phenolic compounds which are found to be devastating against *Leuconostoc mesenteroides*, *Lactobacillus delbruekii*, *Saccharomyces cerevisiae* and *Candida krusei* (Tavassoli and Djomeh, 2011). These polyphenols increases functionality and healthiness of food. Antimicrobial activity of rosemary hydro alcoholic extract was proven against *S. mitis*, *S. sanguinis*, *S. mutans*, *S. sobrinus* and *L. casei* standard strains. Use of rosemary extracts for food preservation not only enhances shelf life but also prevents its economic loss (Silva and Junior, 2010).

LEMON GRASS

Lemon grass (*Cymbopogon citratus* L.) is a perennial herb belongs to poaceae family. Fever grass, citronella grass, silky heads and barbed wire grass are the other names of lemon grass. Lemon grass is grown for culinary and medicinal purpose in Asia and India. Lemon grass oil has antifungal properties and is used as a preservative, pesticide, insect repellent and in soaps. It is also used for the treatment of stomach disorders, malaria and typhoid. Lemon grass oil (LGO), due to its medicinal importance, is used to cure pimples, scabies, headache and circulatory disorder. LGO is used as drug for microbial infections and heals skin related problems. It contains geraniol and citronellol chemical constituents which are active ingredients for household disinfectants and sanitizers. α -citral (geranial) and β -citral (neral) present in oil from lemon grass is responsible for antibacterial activity of microencapsulation of bacteria (gram +ve and gram -ve).

The minimum bactericidal Concentration (MBC) of lemon grass (ethanolic extract) is 128 mg/ml and found to have good bacteriocidal effect on *E. coli*, *S. paratyphi*, *S. flexnerii*, *K. pneumoniae*, *P. aeruginosa* and *B. subtilis* (Fagbemi *et al.*, 2009). Lemon grass oil has strong fungicidal power that inhibits the growth levels of 35, 45 and 47 species of fungus when used at the concentration of 500, 1000, and 1500 ppm. Lemon grass oil is non-phytotoxic and does not affect plant germination and growth. It contains phytochemicals which shows antimicrobial and preservative properties. Lemon grass oil used at 25 ppm inhibits the production of fungal spores up to 70% (Tzortzakis and Economakis, 2007). Studies have shown that LGO is effective antibacterial and antifungal oil at 1 mg/mL concentration. Strains of

Aspergillus spp., *Candida spp.*, *Lactobacillus acidophilus*, *Morganella morganii* and *Penicillium spp.* were tested and found to be sensitive to lemon grass oil (Singh *et al.*, 2011). The antimicrobial activity of lemon grass oil is studied against pathogenic bacteria including *Escherichia coli*, *Bacillus cereus*, *Klebsiella pneumonia*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. It was found that bacteria which are gram positive are more sensitive to LGO as compared to gram negative. In this study activity of lemon grass oil increases with the increase in its concentration and showed highest at 30% Maximum Inhibitory Concentration (MIC) (Naik *et al.*, 2010). Tyagi and Malik (2010) investigated that lemon grass oil (LGO) when used at 288g/ml and 32.7g/ml concentration inhibits the growth of *Candida albicans*.

BASIL

Basil (*Ocimum basilicum*) belongs to Lamiaceae family, is a hard annual plant basically native to India. Basil is used in culinary as fresh or dried herb. It retains its flavor when kept refrigerated or frozen. Thai basil, lemon basil, Sweet basil and holy basil are some of the varieties that are being consumed in Italian food. Oil from basil is characterized by antifungal and insect repellent properties. The chemical components of basil include Citronellol, linalool, myrcene, pinene, terpineol, fenchyl acetate, trans-ocimene, methyl chavicol, eugenol, methyl eugenol and neral. A comparative study has revealed that basil oil plays an antimicrobial role against *Propionibacterium acnes*. This study includes famous culinary herbs, *Ocimum sanctum* L. (holy basil), *Ocimum americanum* L. (hoary basil) and *Ocimum basilicum* L. (sweet basil). Highest growth inhibition of *Propionibacterium acnes* was shown by sweet basil oil at 2.0% v/v conc. and lowest by Holy basil oil at 3.0% v/v conc. (Viyoch *et al.*, 2006). It was investigated that Gram positive bacteria are more susceptible to basil essential oil than to Gram negative bacteria. The chemical composition and the antimicrobial property of Basil are varied by the seasonal variations. A study investigated the antimicrobial property of oil extracted from basil (collected seasonally) against nine pathogens. It was found that samples collected in winter and autumn season shows high antimicrobial activity compared to spring and summer season (Hussain *et al.*, 2008).

CONCLUSION

Consumers have turned out to be more conscious about the prepared food they eat. Synthetic additives, which have been utilized as a flavoring agent for a considerable period of time, may prompt negative

wellbeing results. Additionally, the utilization of food additive have certain disadvantages, for example, expanding cost, taking care of dangers, health hazards and environmental pollution. In this way, the trend is shifting towards supplant natural additives which are low cost, non-hazardous and non-toxic. So in this regard utilization of plant products such as essential oils have been perceived importance since old times. Normal plant items have been utilized for remedial purposes for most of the infectious and non infectious diseases and their utilization is a more noteworthy request now-a-days. Plant essential oils are used as an antiseptic agent since ancient times. As the plant extracts and essential oil are natural products so used for therapeutic purpose. A great number of people rely on plant products because these essential oils are easy to use, cost effective and having no side effects. Plant extract and essential oils are used by most of the population for the administration of different infection diseases, but there is not enough knowledge about how it reacts, safety, toxicity, health hazards and dose. Therefore, it is need of the day to search new, natural, safe, non-toxic and cost effective antimicrobial agents.

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