PAK. J. FOOD SCI., 22(3), 2012: 117-127
ISSN: 2226-5899

Phytochemical characteristics of Date Palm (*Phoenix dactylifera)* fruit extracts

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ABSTRACT

The recent research activities are focused on finding the natural sources of antioxidants as consumers are more conscious about their diet and the synthetic antioxidants are being restricted these days due to their carcinogenicity. So there is more growing trend in searching for antioxidants of natural origin. The present research project was undertaken to explore the antioxidant potential of three Pakistani date varieties. The project was under taken to determine the total phenolics and antioxidant activity by DPPH and β-carotene of non-volatile extracts of three different date vatieties i.e. Dora, Dhakki and Karbalane. Antioxidants of non volatiles of date palm fruit extracts were utilized in a food model system (development of cake) and their potential was evaluated. The methanol extract of Karbaline showed the highest yield (5.01 ± 0.75) and Dora had highest total phenolic content (55.648±0.11 mg/g dry extract). The highest inhibition was attained by the methanol extract of karbaline (90.96%) at 200 µg/mL concentration. Interaction of variety & concentration and solvent & concentration was found significant. The β-carotene assay of Karbaline extract showed highest (98.87%) inhibition. Interaction of variety & concentration and solvent & concentration was also observed significant. Treatments containing date pastes results in lower amounts of TBA products and POV products. All the treatments had similar effect with respect to storage days. Collectively this data emphasize on the chemical composition of different Date verities cultivated in Pakistan and some variation in their antioxidative potential. Our results indicate that the inclusion of both Date fruit and its extracts in the food will increase the content of antioxidants, and thus probably prevent oxidative deterioration of food. So this study concludes that date palm fruit have good antioxidant potential and can be used to produce novel natural antioxidants as well as flavoring agents that can be used in various food products. The paper is useful for food manufactures in order to develop functional foods for consumer. Consumers will prefer the foods rich in antioxidants because they are more conscious about there health and functional food provide them what they desire i.e. health benefits with good nutrition.

Key words: Date Palm, Phenolics, DPPH, Cake, TBA, POV

INTRODUCTION

Date palm *(Phoenix dactylifera L.)* belongs to the Palmae (Arecaceae) family. The Date Palm is a palm extensively cultivated for its edible fruit (Rani *et al.*, 2007). The fruits of the date palm (*Phoenix dactylifera L.*) are sweet berries with a sugar content of more than 50%. Normally, this palm cultivated for local markets on small land holdings besides other. Because of its high nutritional value, great yields and its long life the date palm has been mentioned as the "tree of life" (Augstburger *et al.,* 2002). Date pulps hold easily digestible sugars (70%), mostly glucose, sucrose and fructose; dietary fibers and enclose less proteins and fats (Al Farsi & Lee, 2008). They also enclose vitamins like riboflavin, biotin, thiamine, ascorbic and folic acid that are essential for the body. The pulps of the fruit are rich in calcium, iron, copper, cobalt, magnesium, fluorine, manganese, phosphorus, potassium, copper, sodium, boron, sulfur, zinc and selenium (Al Farsi & Lee, 2008; Ali Mohamed & Khamis, 2004; Elias, 2008). The fleshy tissues of dates contain 0.2-0.5% oil, while the seed contains 7.7-9.7% oil (Walid Al-Shahib & Richard, 2003).

The date fruit pulp is wealthy in phytochemicals like sterols, phenolics, carotenoids, procyanidins, anthocyanins and flavonoids. The concentrations and ratio of these constituents depend on the stage of fruit picking, type of the fruit, location and soil conditions. These phytochemicals also add to the nutritional and organoleptic properties of the fruits (Abdelhak *et al.,* 2005; Abdul & Allaith, 2008). In vitro studies have exposed that the aqueous extract of date fruit is a powerful scavenger of hydroxyl radicals and superoxide and to restrain protein oxidation and iron-induced lipid peroxidation in the rat brain homogenate in a

concentration dependent manner (Vayalil, 2002). Afterward, other investigators have confirmed these explanations with different varieties of date (Abdul & Allaith, 2008; Al Farsi *et al.,* 2005). The existence of a variety of phenolic compounds particularly the coumaric acid and ferulic acid derivatives may have been accountable for the observed free radical scavenging effects.

Dates are especially delicious as a fresh fruit. Beside direct consumption of the whole dates the fruits are traditionally used to prepare a wide range of different products such as date juice concentrates (spread, syrup and liquid sugar), fermented date products (wine, alcohol, vinegar, organic acids) and date pastes for different uses (e.g. bakery and confectionary). Also the by-products arising from date processing can be used for different purposes (Augstburger *et al.,* 2002). When used in baking they provide superb taste to the final product. Dates are also used as a component in food preparations like sweets, snacks, confectionery, baking products, institutional feeding and health foods (Rani *et al.*, 2007).

There is a more growing trend in searching for antioxidants of natural origin. Fresh fruits are an excellent source of natural antioxidants and some of them even outperform the synthetic antioxidants, and are safer also from the health point of view. Date fruit is rich in antioxidants compounds which can be exploited but there is lack of research in the determination of antioxidants from date fruit and its subsequent use as a natural source of antioxidants. So it is of great importance to study the antioxidant activity of local date's varieties and their application in food model to explore it as a natural source of antioxidants. Therefore the present research project was undertaken with the key objectives to determine the total phenolics and antioxidant activity by DPPH and β-carotene of non-volatile extracts of different date varieties and to utilize the antioxidants in food model system. The requisite information when accessible will improve our knowledge and positive reception for the use of dates in our daily diet.

MATERIALS AND METHODS

Procurement of samples and reagents

Dora, Dhakki and Karbalane varieties of date palm fruit were obtained from Date Palm Research Sub-station Jhang, Pakistan. The chemicals and reagents used in the study (n-hexane, methanol, folin-ciocalteu reagent, gallic acid, anhydrous sodium carbonate, 1,1-diphenyl-2-picrylhdrazyl (DPPH), β-carotene, chloroform, linoleic acid and tween 80) were purchased from sigma company USA.

Chemical analysis of date varieties

The samples of three date varieties were analyzed for various chemical characteristics like moisture, fat, ash and protein contents according to the method no 44-15A, 30-10, 08-01 and 46-13 respectively as described in the AOAC (1999).

Extraction of non-volatile compounds

The non-volatile compounds were extracted by solvent extraction method (El-Ghorab *et al*., 1999). Weighed amount of sample was taken in a flask and the flask was filled with the solvent (n-hexane/ methanol). The flask was shaken for 48 hours with 3 hours interval. These extract was filtered through filter paper and subjected to rotary evaporation. Concentrated sample was stored in freezer for further analysis.

Total phenol determination

The total phenolic compounds were estimated by Folin-Ciocalteu method according to Sun *et al*. (2006). The sample solution 125 µL of sample was taken, then 500 µL distilled water was added in it and then 125 µL of Folin-Ciocalteu reagent was added. Then 1.25 mL of 7% sodium carbonate was added in it. The final volume was made 3mL by adding 1mL distilled water and then absorbance of the samples was read in triplicate at 760 nm by using a UV-vis spectrophotometer.

Determination of antioxidant activity

The free radical scavenging activity of date extracts was measured by spectrophotometer at 517 nm by following the method reported by Kim *et al.* (2005). Antioxidant activity was also determined by β-carotene method using spectrophotometer at 470 nm according to the methods as described by Hinnebgurg *et al*. (2006).

Preparation of cake

Cake was prepared by following the method no 10-90 as detailed in AACC (2000). The ingredients used were flour (200g), sugar (280g), shortening (100g), dry milk (24g), dried egg white (18g) and salt (6g).

Following treatments of date paste were used for the preparation of cakes.

Treatment	BHT	Dhakki	Dora	Karbalane
T_1	0.01%	-	-	-
T_2	-	1.50%	-	-
T_3	-	3.00%	-	-
T_4	-		1.50%	-
T_5	-	-	3.00%	-
T_6	-	-		1.50%
T_7	-	-		3.00%

Assessment of lipid oxidation in cake

Measurement of 2-thiobarbituric acid (TBA)

The TBA assay of cakes was carried out at 7 days interval to determine the malonaldehyde produce during storage of the cakes by following the method as described by Schmedes & Holmer (1989). The 2-thiobarbituric acid (TBA) assay was carried out according to the procedure of Schmedes and Holmer (1989). Cake sample (10 g) was mixed with 25 ml of trichloroacetic acid solution (200 g/l of TCA in 135 ml/l phosphoric acidsolution) and homogenized in a blender for 30 seconds. After filtration, 2 ml of the filtrate were added to 2 ml TBA solution (3 g/l) in a test tube. The test tubes were incubated at room temperature in the dark for 20 hours; then the absorbance was measured at 532 nm by using UV–VIS spectrophotometer (model UV-1200, Shimadzu, Japan). TBA value was expressed as mg malonaldehyde per kg of cake.

Peroxide value

The Peroxide value (POV) of cakes was determined at 7 days interval peroxide milli equivalent / kg sample according to the method as described in AOAC International (1999). The sample (3 g) was weighed in a 250-ml glass stoppered Erlenmeyer flask and heated in a water bath at 60°C for 3 min to melt the fat, then thoroughly agitated for 3 min with 30 ml acetic acid–chloroform solution (3:2 v/v) to dissolve the fat. The sample was filtered under vacuum through Whatman filter paper to remove cake particles. Saturated potassium iodide solution (0.5 ml) was added to the filtrate, which was transferred into the burette of an automatic titrator (DL 25 Titrator, Mettler-Toledo AG, Greifensee, Switzerland) equipped with stirrer and pH electrode. The titration was allowed to run against standard solution of sodium thiosulfate (25 g/l). POV was calculated and expressed as milliequivalent peroxide per kg of sample:

$$\text{POV (meq / kg)} = \frac{S \times N \times 1000}{W}$$

Where S is the volume of titration (ml), N the normality of sodium thiosulfate solution (N =0.01) and W the sample weight (kg).

Statistical Analysis

Data is presented as Mean + Standard Deviation; Anova and LSD test to determine the level of significance and to draw conclusions (Steel *et al*., 1997).

RESULTS AND DISCUSSION

Proximate analyses

The result (Table 1) shows that the moisture content for dates is very low. The date variety Dhora showed the highest moisture content which was 14.81 ± 0.396 and Dhaki showed the lowest moisture content (9.90 ± 0.042%). The protein content of Karbaline and Dhaki were 2.7 ± 0.187% and 2.4 ± 0.052% respectively on fresh weight basis. Dora showed crude protein content of 2.1 ± 0.315%. The crude fat content of the Dora samples was 0.4 ± 0.319% which was almost same reported by Ahmad *et al.* (1995) and was observed 0.2%,while Dhaki and Karbaline have very less amount of fat content i.e. 0.2% ± 0.289% and 0.2% ± 0.249, respectively. In case of ash content Dhaki showed highest value i.e. 1.9± 0.297% while Karbaline and Dora have amount of ash content 1.6 ± 0.017 and 1.4 ± 0.171% respectively. These difference may be due difference in climatic and storage conditions.

Extraction of non-volatile compounds

The percent yield of non-volatile extracts on weight/weight (w/w) basis is shown in Table 2. It is evident that the methanol extracts have higher yield as compared to the n-hexane extracts. The methanol extract of Karbaline has the highest yield (5.01 ± 0.75), while n-hexane extract of Dora variety has the lowest yield (0.62± 0.34).

Total Phenolic Content

Total phenolic content of the n-hexane and methanol extracts of date samples was measured by using Folin's reagent. The results (Table 3) have been presented as mg of gallic acid equivalent (GAE) per gram of dry extract. It was shown that methanol and n-hexane extracts of Dhaki variety had the phenolic contents up to 4.866±0.115 and 2.659±0.340, respectively which were in accordance with the results of AL-Farsi *et al.* (2005), Biglari *et al.* (2009) and Ameer & Allaith (2008). It was also shown that methanol extract of Dora variety had highest total phenolic content that reached 55.648±0.11 mg/g dry extract. Similarly n-hexane extract of same variety also showed high total phenolics which was 3.309±0.133. These results agree to a previous study in which AL-Farsi *et al. (*2005), Biglari *et al.* (2009) and Allaith and Ameer (2008) studied the antioxidant activity of the methanol extracts of dates and estimated its phenolic content which was 2.490±.01 to 8.36±0.60 mg GAE/g dry weight of the sample. Literature showed that antioxidants activity is directly related to total phenolic contents. It means the sample having the higher total phenolic contents will show the higher antioxidant activity. A significant correlation existed between antioxidant activity and total phenolics in our study.

Antioxidant activity

In this study the antioxidant activity of the non-volatile components of three native varieties of dates (n-hexane and methanol extracts) was determined by using DPPH and β-carotene method. The results were compared with the synthetic antioxidant BHT which is an efficient synthetic antioxidant agent in food.

1, 1-diphenyl-2-picrylhdrazyl (DPPH) scavenging activity

In this research, different concentrations of the non-volatile components (n-hexane and methanol extracts) of non-volatile components of dates varieties were treated with DPPH radical, starting from 50 µg/mL to 200 µg/mL and effect of these concentrations on the inhibition of the DPPH radical was studied. The highest inhibition was attained by the methnol extract of karbaline variety which was 90.96% when concentration of sample 200µg/mL was used (Table 4). The low inhibition percentage of the hexane extract of dora variety was 60.42% when 50µg/mL concentration was used. This may be due to the oxidation of some antioxidant compounds of the extracts or may the low concentration of the extract. The methanol extract of dhaki dates also showed higher scavenging effect which was 85.75% when concentration of sample 200µg/mL was used and hexane extract of same variety had highest value which was 77.68%.

Interaction of variety and concentration was also significant. The interactive effect of the solvents and concentrations are shown in the Table 5. The high inhibition was attained by the extract of karbaline variety which was 80.81% when concentration of sample 50µg/mL was used. The lowest inhibition percentage of dora variety was 70.60% when 200µg/mL concentration was used. The extract of dhaki dates also showed higher scavenging effect which was 80.07% when concentration of sample 50µg/mL was used and extract of same variety had highest value which was 83.89% at 200µg/mL.The mean values for all concentration did not show much difference among themselves.

Incase of interaction between solvent and concentration which was also significant and shown in the Table 6. The high inhibition was attained by the extracts of methanol at all concentrations. The lower inhibition percentage was observed for all concentrations of hexane. The highest scavenging effect was 89.18% for methanol when concentration of sample 100µg/mL was used and the lowest value 67.63 % at same concentration for hexane. The mean values for all concentration did not show much difference among themselves. The mean values for solvents showed considerable difference of about 20%.

β-caroteen bleaching assay

The antioxidant activity was also determined by using β-caroteen bleaching assay. The methanol and hexane extracts were used for determination of β-caroteen bleaching assay. Both the n-Hexane and Methanol extracts were used at the same concentration level 50-200 µg/mL for the determination of antioxidant activity by using β-caroteen bleaching assay. The β-caroteen bleaching mechanism is a free radical-mediated phenomenon, resulting from hydroperoxides formed from linoleic acid. In this study, β-caroteen undergoes Values carrying same letters are non significantly different with each other rapid discoloration due to the attack of free radicals formed upon abstraction of a hydrogen atom from the diallylic methylene group of linoleic acid. The presence of an antioxidant in the reaction mixture hinders the rate of bleaching by neutralizing free radicals formed in the system during incubation at 50^{o}C (Wettasinghe *et al.*, 1999).

Table 7 shows that solvent have significant effect upon the β-carotene bleaching assay, and furthermore the combined effect of variety and solvent has also significant effect on the inhibition of free radical. But there is little bit less effect of all three factors like concentration, solvent and variety. Table 7 showed clear picture that of methanolic extract of Karbaline has maximum inhibition effect than that of other methanolic extracts. There is very less difference between the Karbaline and Dora of methanolic extracts. The Karbaline extract have 98.87% inhibition activity while the Dora have 97.78%.

Table 1 Proximate analysis of date varieties

	Dora	Dhaki	Karbaline
Moisture %	14.81 ± 0.396	9.90 ± 0.042	12.3 ± 0.242
Crude Fat %	0.4 ± 0.319	0.2% ± 0.289	0.2%±0.249
Ash %	1.4 ± 0.171	1.9± 0.297	1.6 ± 0.017
Crude Protein %	2.1 ± 0.315	2.4 ± 0.052	2.7 ± 0.187

The results are presented as Mean ± Standard Deviation (SD)

Table 2 Extraction yield of non-volatile compounds by using different organic solvents

Sample	Yield % w/w ± SD
Hexane Dora extract	0.62 ± 0.34
Hexane Dhaki extract	0.78 ± 0.25
Hexane Karbaline extract	0.93 ± 0.41
Methanol Dora extract	4.08 ± 0.23
Methanol Dhaki extract	4.79 ± 0.82
Methanol Karbaline extract	5.01 ± 0.75

w/w = on weight/ weight basis, SD = Standard Deviation, Values are expressed as mean±SD

Table 3 Total phenolic contents of hexane and methanol extracts of non-volatile compounds

Sample	Phenolic Content (mg / g dry extract)
Methanol Dora	5.648±0.11
Methanol Dhaki	4.866±0.115
Methanol Karbaline	5.144± 0.198
Hexane Dora	3.309± 0.133
Hexane Dhaki	2.659±0.340
Hexane Karbaline	2.288± 0.1499

SD= Standard Deviation, Values are expressed as mean±SD

Table 4 Means for antioxidant activity by DPPH of variety x solvent

Solvents	Antioxidant Activity			Means
	Karbaline	Dhaki	Dora	
Methanol	90.96a	85.75b	89.79a	88.84a
Hexane	69.57d	77.68c	60.42e	69.22b
Means	80.27a	81.71a	75.11b	

Values carrying same letters are non significantly different with each other

Table 5 Means for antioxidant activity by DPPH for variety x concentration

Concentration µg/mL	Antioxidant Activity			Means
	Karbaline	Dhaki	Dora	
50	80.81bc	80.07bcd	78.90cd	79.93a
100	78.22de	81.18bc	75.83e	78.41b
200	81.77ab	83.89a	70.60f	78.75ab
Means	80.27a	81.71a	75.11b	

Values carrying same letters are non significantly different with each other

Table 6 Means for antioxidant activity by DPPH for solvent x concentration

Solvents	Antioxidant Activity			Means
	50 µg/mL	100 µg/mL	200 µg/mL	
Methanol	88.62 a	89.18 a	88.70 a	88.84 a
Hexane	71.24 b	67.63 c	68.80 c	69.22 b
Means	79.93a	78.41b	78.75 ab	

Table 7 Means for antioxidant activity by β- carotene for variety*solvent

Solvents	Antioxidant Activity			Means
	Karbaline	Dhaki	Dora	
Methanol	98.87a	95.32b	97.78a	97.32a
Hexane	79.76d	87.51c	70.55e	79.27b
Means	89.32b	91.42a	84.16c	

Values carrying same letters are non significantly different with each other

Table 8 Means for Antioxidant activity by β-carotene for Variety x Concentration

Concentration µg/mL	Antioxidant Activity			Means
	Karbaline	Dhaki	Dora	
50	89.63 bcd	89.80bcd	88.40cd	89.28a
100	87.55d	91.02ab	84.71e	87.76b
200	90.77bc	93.43a	79.38 f	87.86ab
Means	89.32b	91.42a	84.16c	

Values carrying same letters are non significantly different with each other

Table 9 Means for Antioxidant activity by β-carotene for Solvent x concentration

Solvents	Antioxidant Activity			Means
	50 µg/mL	100 µg/mL	200 µg/mL	
Methanol	97.30a	97.84a	96.82a	97.32a
Hexane	81.25b	78.89c	77.67c	79.27b
Means	89.28a	87.76b	87.86ab	

Values carrying same letters are non significantly different with each other

The n-Hexane extracts have lower inhibition activity, Dora have very less which is 70.55% and the maximum Interaction of variety and concentration was also significant. The interactive effect of the solvents and concentrations are shown in the Table 8. The high inhibition was attained by the extract of karbaline variety which was 90.77% when concentration of sample 200 µg/mL was used. The lowest inhibition percentage of Dora variety was 79.38% when 200 µg/mL concentration was used. The extract of dhaki dates also showed higher scavenging effect which was 89.80% when concentration of sample 50 µg/mL was used and extract of same variety had highest value which was 93.43% at 200 µg/mL.The mean values for all concentration did not show much difference among themselves.

Incase of interaction between solvent and concentration which was also significant and shown in the Table 9. The high inhibition was attained by the extracts of methanol at all concentrations. The lower inhibition percentage was observed for all concentrations of hexane. The highest scavenging effect was 97.82 % for methanol when concentration of sample 100µg/mL was used and the lowest value 77.67 % at 200µg/mL concentration for hexane. The mean values for all concentration did not show much difference among themselves. The mean values for solvents showed considerable difference of about 20%.

The interaction tables also showed that the mean value of methanol extracts has greater inhibition effect than the mean value of n-hexane extracts. There is almost 20% difference between them. All interactions have same trend as in DPPH test.

Assessment of lipid oxidation

TBA value

Lipid peroxidation is one of the crucial mechanisms of quality deterioration. It also results in the development of potentially compounds, among them oxidized a cholesterol derivative which is described by Paniangvait *et al*. (1995). Lipid peroxidation is measure in the form of malondialdehyde compounds from during auto-oxidation of lipid present in meat tissue. Higher the malondialdehyde compounds concentration revealed higher amount of lipids which deteriorate the meat tissue. TBA value is indicator of oxidative deterioration. It is significantly affected with the treatments and storage periods. Interactive effect of both factors affected the TBA significantly for Daki hexane extract which was 87.51% as also showed in DPPH assay while Karbaline have 79.76%.

According to Table 10 cake samples containing BHT produced least amounts of TBA products (0.13) when the sample having BHT stored for 7 days. Treatments containing date pastes results in lower amounts of TBA products. Among treatments T_3 Dakki 3% gave best results (0.94) after 28 days storage period, while among cake samples containing 3% karbaline paste showed good result in treatments T_7 after 28 days storage period. Interestingly TBA gave maximum value which was 0.210. Storage has profound effect on TBA value and it increased with increase in storage and least TBA value was observed and while maximum TBA value was obtained at 28 days of storage. Interactive effects revealed that in TBA value samples with BHT ranged from 0.14 to 0.21. In T_2 TBA value increased from 0.23 to 0.98 and these trends were statistically alike in T_3 and showed better performance after the storage duration and TBA value increased from 0.23 to 0.94.Remaining treatments showed similar response.

Peroxide value

Fats and oils are composed of unsaturated and saturated fatty acids which are susceptible to oxidation when exposed to light, moisture and heat. Peroxide value is a measure of the oxidative rancidity of oil. Oxygen can add to the fatty acid chain to form peroxide and hydroperoxide. Peroxide undergoes cleavage to produce bad smelling aldehydes, ketones and acids. These products interfere seriously with rancidity, discoloration, vitamin destruction, nutritional losses and polymerization making them unacceptable to the consumer. The peroxide value is the measure of the amount of these products and is expressed as the milliequivalent of peroxide oxygen combined with one kilogram of fat/oil.

According to Table 11 cake samples containing BHT produced least amounts of POV products (6.73) when the sample having BHT stored for 7 days. Treatments containing and date pasts results in lower amounts of POV products. Among treatments T_4 gave best results (18.96) after 28 days storage period, while among cake samples containing showed the good result in treatments T_6 and T_7 after 28 days storage period gave maximum POV value which was19.33.All treatments had similar effect with respect to storage days.

Table 10 Means for TBA value of cake samples containing the date paste

Treatment	Storage Intervals				Mean
	7 days	14 days	21 days	28 days	
$T_{1\ BHT}$	0.13h	0.48cdef	0.183 h	0.210 h	0.25b
T_2	0.23h	0.46ef	0.62bcde	0.98a	0.57a
T_3	0.23h	0.42fj	0.65bc	0.94a	0.56a
T_4	0.24gh	0.46def	0.68b	0.97a	0.59a
T_5	0.25gh	0.46def	0.67b	0.97a	0.59a
T_6	0.24gh	0.45ef	0.94bcd	0.96a	0.57a
T_7	0.25gh	0.46ef	0.69b	0.96a	0.59a
Mean	0.22d	0.45c	0.95b	0.85a	

Values carrying same letters are non significantly different with each other

Table 11 Means for peroxide value of cake samples containing the date paste

Treatment	Storage Intervals				Mean
	7 days	14 days	21 days	28 days	
$T_{1\ BHT}$	6.73i	9.40h	13.13e	16.1bc	11.34c
T_2	6.66i	11.44f	16.03bcd	18.96a	13.27ab
T_3	6.46i	10.70g	15.50cd	19.13a	12.95b
T_4	6.60i	11.33fg	15.46cd	18.96a	13.09ab
T_5	6.70i	11.23fg	16.50b	19a	13.35a
T_6	6.80i	11.33fg	16.06bc	19.33a	13.38a
T_7	6.73i	11.16fg	15.3d	19.33a	13.13ab
Mean	6.67d	10.94c	15.42b	18.69a	

Values carrying same letters are non significantly different with each other

Storage has profound effect on POV value and it increased with increase in storage and least POV value was observed at 7 days and while maximum POV value was obtained at 28 days of storage. Interactive effects revealed that in POV value samples with BHT ranged from 6.73 to 16.10. In T_2 POV value increased from 6.66 to 19.33 and these trends were statistically alike in T4 while remaining treatments showed identical performance throughout the storage duration and POV value increased from 5.70 to 17.69. In the present study, POV in all samples were below 25meq of active O_2/kg, which is considered as limit of acceptability in fatty foods (Evranuz, 1993).

CONCLUSION

Collectively this data emphasize on the chemical composition of different Date varieties cultivated in Pakistan and some variation in their antioxidative potential. In this study we have shown the variety, solvent and concentration dependent inhibitory activity towards radical scavenging properties. The results are more potent in methanol extracts as compared to hexane extracts. Nutritionally, date fruit is a good source of energy and a mixture of antioxidants including ascorbic acid, carotenoids, flavonoids and polyphenols it is essential that compositional studies in plant foods be carried out to take into account various factors such as cultivars, seasons and pre- and post-harvest conditions that may affect the chemical composition of plant foods. Methanol extracts of all the samples were found to have better antioxidant action than the n-hexane extracts. There was also found a good correlation between the total phenolic content and antioxidant activities of the nonvolatile extracts. Our results indicate that the inclusion of both Date fruit and its extracts in the food will increase the content of antioxidants, and thus probably prevent oxidative deterioration of food. It seems that several different compounds mediate antioxidant activity. So this study concludes that date palm fruit have good antioxidant potential and can be used to produce novel natural antioxidants as well as flavoring agents that can be used in various food products. Due to its low cost and abundance, dates remain a species with incredible potential and innumerable possibilities for further investigation.

REFERENCES

1. Abdul, A., & Allaith, A. (2008) Antioxidant activity of Bahraini date palm (Phoenix dactylifera L.) fruit of various cultivars. *International Journal of Food Science and Technology,* 43, 1033–1040.
2. Ahmed, I.A., & Ahmed, A. (1995). Chemical composition of date varieties as influenced by the stage of ripening. *Food Chemistry*, 54, 305-309.
3. Al Farsi, M. A., & Lee, C. Y. (2008). Nutritional and functional properties of dates: a review. *Critical Reviews in Food Science and Nutrition*, 48, 877–887.
4. Al Farsi, M., Alasalvar, C., Morris, A., Baron, M., & Shahidi, F. (2005). Compositional and sensory characteristics of three native sun-dried date (Phoenix dactylifera L.) varieties grown in Oman. *Journal of Agricultural and Food Chemistry,* 53, 7586–7591.
5. AL-Farsi, M.C., Alasalvar, A., Morris, A., Baron, M., & Shahid, F. (2005). Comparison of Antioxidant Activity, Anthocyanins, Carotenoids, and Phenolics of Three Native Fresh and Sun-Dried Date (*Phoenix dactylifera* L.) Varieties Grown in Oman. *Journal of Agricultural and Food Chemistry,* 53, 7592-7599.
6. Ali Mohamed A.Y., & Khamis, A. S. (2004). Mineral ion content of the seeds of six cultivars of Bahraini date palm (Phoenix dactylifera). *Journal of Agricultural and Food Chemistry,* 52, 6522–6525.
7. Ameer, A., & Allaith, A. (2008). Antioxidant activity of Bahraini date palm (*Phoenix dactylifera L.*) fruit of various cultivars. *International Journal of Food Science and Technology,* 43, 1033–1040.
8. American Association of Cereal Chemists. (2000). Approved Methods of the American Association of Cereal Chemists, 10th Ed., Inc. St. Paul, MN.
9. Association of official analytical chemists. (1999). Official methods of analysis of AOAC international (16th ed.). Gaithersburg MD. USA
10. Augstburger, F., Berger, J., Censkowsky, U., Heid, P., Milz, J., & Streit, C. (2002). Date Palm. Naturland. Germany.
11. Biglari, F., Abbas, F. M., AlKarkhi, M. E., & Azhar. (2009). Cluster analysis of antioxidant compounds in dates (*Phoenix dactylifera*). Effect of long-term cold storage. *Food Chemistry,* 112, 998–1001.
12. El-Ghorab, A., Fadel, H., Marx, F., & El-Sawy, A. (1999). Effect of extraction techniques on the chemical composition and antioxidant activity of *Eucalyptus camaldulensis* Var. brevirostris leaf oils. *Z. Leb. Unt. For. A.* 208, 212-216.
13. Elias, Y. M. (2008). Date - A Wonder Fruit of Arabia. Retrieved April 1, 2009, from

http://ezinearticles.com/?Date---A-Wonder-Fruit-of-Arabia&id=1527461.

14. Evranuz, O.E. (1993). The effects of temperature and moisture contenton lipid peroxidation during storage of unblanched salted roasted peanuts: shelf life studies for unblanched salted roasted peanuts. *International Journal of Food Science and Technology,* 28: 193–199.
15. Hinneburg, I., Dorman, H. J. D., & Hiltunen, R. (2006). Antioxidant activities of extracts from selected culinary herbs and spices. *Food Chemistry,* 97, 122-129.
16. Kim, H., Chen, Y. F., Wang. Z., &.Rajapakse. N. (2005). Effect of chitosan on the biological properties of sweet basil. *Journal Agricultural and Food Chemistry*, 53, 3696–3701.
17. Paniangvait, P., King, A. J., Jones, A. D., & German, B. G. (1995). Cholesterol oxides in foods of animal origin. *Journal of Food Science,* 60, 1159–1174.
18. Rani, C. I., Kalaiselvi, T., & Jegadeeswari, V. (2007). The date palm. Accessed at www.techno-preneur.net/information-desk/.../2007/.../Date_palm.pdf
19. Schmedes, A., & Holmer, G. 1989. A new thiobarbituric acid (TBA) method for determination of free malonaldehyde (MDA) and hydroperoxides selectivity as a measure of lipid peroxidation. *Journal of American Oil Chemist Society*, 66, 813–817.
20. Steel, R.D., Torrie, J. H., & Dicky, A. (1997). Principles and procedures of Statistics. A Biometrical Approach 3rd Ed. McGraw Hill Book international Co., Singapore. pp. 204-227.
21. Sun, T., Xu, Z., Wu, C. T., Janesm, M., & No, H. K. (2006). Antioxidant activities of different colored Sweet Bell Pepper (*Capsicum annum* L.). *Journal of Food Science*, 72, 98-102.
22. Vayalil, P. K. (2002). Antioxidant and antimutagenic properties of aqueous extract of date fruit (Phoenix dactylifera L. Arecaceae). *Journal of Agricultural and Food Chemistry*, 50. 610–617.
23. Walid Al-Shahib & Richard, J. M. (2004). The fruit of the date palm its possible use as the best food for the future. *International Journal of Food Science and Nutrition*, 54, 247 -259.
24. Wettasinghe, M., & Shahidi, F. (1999). Evening Prime Rose Meal. A source of natural antioxidants and scavenger of hydrogen peroxide and oxygen derived free radicals. *Journal Agricultural and Food Chemistry*, 47, 1801-1812.

PAK. J. FOOD SCI., 22(3), 2012: 128-132
ISSN: 2226-5899

Cooking and eating characteristics of Rice (*Oryza sativa* L.)-A review

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Abstract

Rice is staple food of Pakistani inhabitants and is a source of foreign exchange earnings. It is an immense source of starch. Rice starch is digested so quickly than any other high starchy food and this aspect make it distinctive among other cereals. The cooking and eating value is determined by the amylose content and gelatinization temperature. The cooking and eating characteristics of rice is the base of choice for the consumers. The amylose content is great determinant of rice cooking and eating characteristics. The introduction of newly evolved rice cultivars and high yielding rice varieties have enhanced the rice yield to a great extent, but no or little emphasis has been given to evaluate the cooking and eating characteristics of Pakistani rice varieties. The present literature was reviewed to analyze the physicochemical properties, cooking and eating characteristics and amylose content of different rice cultivars.

Keywords: Rice, starch, amylose, gelatinization, basmati

Physical and chemical changes occur during the rice storage. The eating and cooking properties are affected by the starch, protein and protein interaction, only structural changes occur rather than the change in the starch and protein interactions. These structural changes affect the flavor, texture, gelling and pasting characteristics. The range for milling of rice varieties was 64-70% and head rice out-turns range was 82%. The rice variety Superfast showed the highest milling out-turns that was nearly 70% while the lowest was observed for Khazar (Zhou *et al*., 2002)

Rice is an enormous source of starch and it is the component which affects the cooking and eating properties of rice. Rice starch is digested so quickly than any other high starchy food and this aspect make it distinctive among other cereals. The cooking and eating value is determined by the amylose content and gelatinization temperature. The cooking and eating characteristics of rice is the base of choice for the consumers. The amylose content is great determinant of rice cooking and eating characteristics. Amylose content is significantly affected by the various storage intervals and treatments. The amylose content of Basmati Super (222.91) and Basmati-385 (21.97) indicated a significant difference between the amylose content of Basmati Super (222.91) and Basmati-385 (21.97). An increase in gelatinization temperature has been observed with the decrease in alkali spreading value of rice starch.. Basmati-385 had 3.75 for alkali spreading value while Basmati Super has mean value 4.27. The cooked rice texture perceived by the consumer governs the rice receiving. It is a multidimensional characteristic and palatability of rice is governed by these textural characteristics. The rice variety, amylose content, gelatinization temperature and cooking methods are the factors affecting the rice texture. Rice texture is soft and sticky for varieties having low amylose content while rice varieties become stiff and fluffy on cooking having high amylose content (Shabbir, 2008).

The grain size and shape were explained by the scale for size category. Cooking and eating characteristics included the grain elongation, amylose content, gel consistency, gelatinization temperature and aroma (Khush *et al*., 1979).

Rice varieties according to round, medium or long shape as well as by the ratio of length to width ratio. The shape of Indica rice varieties is wider and dense while grains of Japonica are more in length wise arrangement and slender in configuration. The classification has been suggested on the basis of cooking properties and final gelatinization temperature. The grains of japonica type cook non-sticky and well – separated while the grains of indica type become

soften on cooking and become mashy (Kent, 1982).

The six rice varieties showed the similar morphological appearance, but differ in amylose content and pasting characteristics. The structure of wild rice amylopectin was near to the waxy rice amylopectin possessing more branching and a large proportion of short branch chains. The difference in physicochemical characteristics of six wild rice varieties was due to the difference in branch chain length distribution of amylose and amylopectin (Wang and Porter, 2002).

Starch is the most important factor in rice, therefore, rice texture is significantly affected by the gelatinization and retrogradation of starch. Quite minor and medium gelatinization temperature has been observed in short and medium grain cultivars (Fan *et al*., 1999).

The tenderness and stickiness of the rice kernel is determined by the cooking time. The rice imbibition ratio is inversely related with the weight of cooked rice. The consumers of urban areas prefer rice which expands more in length wise than breadth wise on cooking while the working class consumers are not so much conscious about the rice whether it expands more in length wise arrangement or breadth wise (Denials *et al*., 1998).

The cooking quality of rice is influenced by the gelatinization and retrogradation characteristics of its starch. The range for high amylose containing rice was generally from 15-35%. The rice grains become dry and become firm upon cooling. The rice varieties having low amylose content cook wet and sticky. In major rice producing areas of the world intermediate amylose contents of rice is like most. There are many factors affecting the physicochemical properties of rice starch. The physicochemical properties are manipulated broadly by the varieties, composition and structure, processing method and storage conditions of rice starch. On other hand, many factors like rice cultivars, moisture content, proteins content, lipid, amylose content, processing methods, prolamin, pH affect the amylose content of rice (Zhout *et al*., 2001; Zhou *et al*., 2003).

The physicochemical properties such as pasting properties and gel vigor are the most significant characteristics. Starch is the principal component in rice due to which gelatinization of starch significantly affects the properties of cooked rice. The milled rice contain starch as the major constituent and its features differ broadly among different rice cultivars as depicted by the amylose: amylopectin ratio and final gelatinization temperature. Amylose is considered as major factor responsible for the functional changes. The pasting properties rice starch granules are governed by the rigor of starch granules, which in turn affects the swelling potential of rice starch granules (Juliano, 1990).

The cooking and eating characteristics of rice starch are controlled by the rice starch source, genotype and amylose: amylopectin ratio. Short term as well as long term storage has effect on rice pasting as well as cooking and eating characteristics but the long term storage has significant effect (Perdon *et al*., 1997).

The rice starches with high amylose: amylopectin ratio take up more water during boiling and are considered more desirable for cooking purpose. Amylose content affect the cooking and eating properties and difference in rice varieties such as grain whiteness, grain shape. The rice cooking and eating properties are greatly subjective to Gelatinization temperature and amylose content. Gelatinization temperature which is very important test to determine the cooking quality of rice. Its range varies from 55°C to 79°C (Chrastil *et al*., 1992).

The high amylose content is correlated to the high volume expansion ratio and flakiness of rice. High AC in rice grains causes rice to become dried, decrease in softness and hard upon cooling in contrast to low amylose content. Higher peak viscosity has been observed for the stored rice than fresh rice. Ageing reduces the ability of starch granules to crack and split open after cooking to a large extent but the final viscosity and set back increases with the increase in storage time. Storage increases the rice hardens but stickiness decreases. The cooking and eating characteristics are greatly determined by its gelatinization characteristics. Rice viscosity increases with the increase in storage. The peak viscosities of rice decreases after storage as measured by amylograph, it was lower as compared to fresh rice and the same trend was observed for the final viscosities. The decrease in the peak viscosities of rice starch granules showed that the stored rice showed more resistance than the freshly harvested rice. The increase in peak viscosity was observed up to 6 %, setback value was 33 % and final viscosity was 19%. Zhout *et al*., (2001), described that the

cooking quality of rice is influenced by the gelatinization and retrogradation characteristics of its starch (Zhou *et al*., 2003).

The range for high amylose containing rice was generally from 15-35%. The rice grains become dry and become firm upon cooling. The rice varieties having low amylose content cook wet and sticky. In major rice producing areas of the world intermediate amylose contents of rice is like most. There are many factors affecting the physicochemical properties of rice starch. The physicochemical properties are manipulated broadly by the varieties, composition and structure, processing method and storage conditions of rice starch. On other hand, many factors like rice cultivars, moisture content, proteins content, lipid, amylose content, processing methods, prolamin, pH affect the amylose content of rice (Lai, 2001), stated that the milling methods, storage conditions freezing and melting conditions direct the physicochemical properties of rice starch.

The variation has been observed in the aroma of recently reaped and warehoused rice grain as well as its volume expansion and elongation ratio also showed variation. The amylose: amylopectin ratio of rice is the central property of rice starch and it is important parameter to determine the eating and cooking characteristic Rice starches with high amylose: amylopectin ratio take up more water during boiling and are acknowledged extra pleasant for eating and cooking purpose. The rice varieties showed distinction in amylose content. The composition of the volatile compounds of rice and rice aroma was not contributed by any single volatile compound, however, 2–acetyl-1-pyrroline it is the most donating compounds for its contribution towards aroma in rice varieties (Singh *et al*., 2006).

The cooking and pasting characteristics of rice starch in Asian rice varieties. The milled rice samples of five cultivars were studied for their protein and amylose content and its range was 5.74 to 10.98% and 5.74 to 10.98% respectively. The range for peak viscosities was 510 to 1085 Brabender Units (BU), Final viscosities at 95°C were 40 to 635 BU and set back values were -405 to -620 BU. (Juliano *et al*., 1992),

Rice eating and cooking quality is predicted by the amylose content which is the single main vital factor. The amylose operate as diluent as well at the same time as an inhibitor of swelling of rice starch granules. The amylose content method is more precise to point out the difference in cooking quality of different rice varieties. The variation among rice varieties and their pasting properties is greatly affected by starch and water concentration, protein and operating conditions of the experimental instrument (Batey *et al*., 2000).

Cooked rice texture which governs the rice reception is perceived by the consumers. Rice texture is a multidimensional characteristic and firmness and gumminess of rice is significant and palatability of rice is governed by these textural characteristics. There are number of factors affecting the rice texture including the variety, amylose content, and cooking methods. The rice characteristics are significantly influenced by the gelatinization and retrogradation of starch as starch is the leading factor which has significant influence on these properties. Relatively low gelatinization temperature has been observed for short and medium grain cultivars. The gelatinization and retrogradation properties of rice are significantly subjected by the temperature, time interval and moisture content during storage. The variation in amylose content was 7.83 to 18.86% showed by different rice cultivars. The amylose content in five cultivars has been separated and it was lowest in PR-103 which is 7.83%, the highest amylose content was shown by the PR-113, PR-114 possess intermediate amylose content, PR-114 contain 16.13% and IR-8 has 15.83%. The starch granules swell upon heating and the amylose content within the granules leaches out concurrently. A three dimensional network develops from the leached amylose. The paste is formed by the gelatinizing the aqueous suspension of starch. The high starch concentration causes the paste to be settled into gel rapidly (Bergman *et al*., 2000),

The amylose content of rice varieties and milling fractions of brown rice was 22.90% to 26.19% and for white rice varieties 24.14% to 25.31%. IIRI-6 contains higher content of amylose and followed by the KS-282 and Super Basmati. The milling fraction of the IRRI-W (26.81%) contains the highest amylose followed by KS-W (26.69%) and IRRI-B contains 25.46%, KS-B contain 25.34%, 23.41% for B2-W, 23.37% for SB-B and the B2-B hold the lowest amylose contents. The amylose content in the range of 18.60-28.0% for different rice varieties (Shabbir *et al.*, 2006).

Amylose content varied from 18.6 to 26% for Basmati varieties showed lower amylose content. The most varieties showed 3.1 to 7.0% alkali spreading value. Water uptake is positively correlated to the alkali spreading value and it ranged from 172-450%. Protein content showed positive correlation with the elongation ratio of rice and it ranged from 6.01 to 10.26% (Chordhurym and Ghosh, 1979).

The varietal properties of rice such as grain size, shape and percentage hull, dormancy and bulk density to a great extent influence the grain quality. The increase in paste viscosity is governed by the starch when it is cooled, this results in the swallowing of starch granules to be ruptured when held at high temperature and subjected to permanent shearing action and it is measured by Breakdown viscosity, degree of retrogradation is measured by setback viscosity. The starch content of milled rice become swollen on cooking by absorbing moisture and some solid content is also dissolved on cooking due to the gelatinization of the starch granules. Rice elongation ratio has a significant relationship with other cooking characteristics. There exist a strong positive correlation with kernel length and with length to breadth ratio and alkali spreading value, water uptake ratio and negative relationship exist with kernel breadth after cooking, gel consistency, optimum cooking time and gelatinization temperature. Positive correlation exist among optimum cooking time with water uptake ratio and kernel breadth after cooking kernel length and breadth after cooking and negative correlation with elongation ratio and cooking time (Juliano, 1990)

High amylose content has been experienced with the coarse rice varieties which are closely interconnected to the property of dry on cooking and less gentleness IIRI-6 has highest FV95°C, 527.0 BU and Basmati 2000 has 453.0 FV95°C, which is lower FV95°C of KS-282 showed 517.0 BU and Super Basmati (SB) varieties showed 472.0 BU, respectively. The highest FV95°C was observed in milling fraction of IRRI-W 545.0 BU and in B2-B. The CV50°C for brown rice of milling fraction is 826.75 and 900.75 BU for white rice. IRRI –W contain highest CV50°C for 992.0 BU and KS (973.0 BU), IRRI- B (879.0 BU), KS-B 859.0 BU, SB-W (833.0 BU), B2-W (805.0 BU), B2-B (774.0 BU) and lowest was found in B2-B (774.0 BU). Storage temperature and treatments greatly affect the volume expansion ratio. The water absorption ratio is determinant of rice eating and cooking quality. Two rice varieties were observed for the affect of treatment and storage intervals. The elongation ratio is greatly determined by the storage interval and treatment (Shabbir *et al*., 2006).

Conclusion

The cooking and pasting characteristics of different rice varieties varied among the rice varieties. This variation also exists among the physical, chemical and sensory characteristics between rice cultivars. The difference in these parameters can be exploited by the rice breeders in their hybridization programme. The better quality rice is also delighted by the consumers.

References

1. Batey, I. L. and B. M. Curtin. 2000. Effects on pasting viscosity of starch and flour from different operating conditions for the Rapid Visco-Analyzer. Cereal Chem.77, 754-760.
2. Bergman, C.J., J. T. Delgado, R. Bryant, K. R. Cadwallader and B. D. Webb.2000. Rapid gas chromatographic technique for quantifying 2-Acetyl-1-Pyrrolone and Hexanal in rice (*Oryza sativa*, L). Cereal Chem. 77, 454-458.
3. Chrastil, J. and Z. M. T. Zarins. 1992. Influence of storage on peptide subunit of Rice. Oryzenin. J. Agri. Food Chem. 40, 927-930.
4. Daniels, M.J., Marks B. P., T. J. Siebenmorgen, R. W. McNewand J. F. Meullenen. 1998. Effects of long-grain rough rice storage history on end-use quality.J. Food Sci. 63, 832-835.
5. Fan, J. and B. P. Marks. 1999. Effects of rough rice storage conditions on gelatinization and retrogradation properties of rice flour. Cereal Chem.76, 894-897.
6. Indudhara, S., Y.M.Sowbhagya and Bhattacharya, K.R.1978.Changes in the physicochemical properties of rice with ageing. J. Sci. Food Agri. 29,627-639.
7. Juliano, B. O. 1990. Criteria and tests for rice grains qualities. Pages 445-490. In, Rice Chem. and Tech. 2nd Ed. Am. Assoc. Cereal Chem. Incorporated, St. Paul, Minn., USA.
8. Juliano, B. O. and C. M. Perze. 1990. Result of a collaborative test on the measurement of grain elongation of milled rice during cooking. J. Cereal Sci. 2, 281–287.
9. Kent, N. L. 1982. Technology of cereals with special reference to wheat.20-21. Pergamon Press, New York.

10. Khush, G. S., C.M. Paule and N. M. Cruz.1979. Rice grain quality evaluation and improvement at IRRI. Proceedings of the workshop on the chemical aspects of rice grain quality. Int. Rice Res. Inst., Philippines. 21-31.
11. Lai, H. M. 2001. Effects of hydrothermal treatment on the physicochemical properties of pregelatinized rice flour. Food Chem.72, 455-463.
12. Perdon, A. A., B. P. Marks, T. J.Siebenmorgen and N. B. Reid. 1997. Effects of rough rice storage conditions on the amylograph and cooking properties of medium-grain rice Bengal. J. Cereal Chem. 74, 864-867.
13. Shabbir, M. A. 2008. Biochemical and technological characteristics of Pakistani rice and protein isolates, Ph.D, Thesis, Dept. Food Tech. Uni. Agri. Fsd.
14. Singh, R. K., U. S. Singh and G. S. Khush. 2006. Rice grain quality evaluations procedure. Food Sci. Biotech.18, 1403-1407.
15. Wang, L., and R. Porter. 2002. Structures and physicochemical properties of six wild rice starches. J. Agri. Food Chem. 50, 26-72.
16. Zhou, Z., K. Robards K., Helliwell S. and Blanchard C. 2002.Ageing of stored rice: Changes in chemical and physical attributes. J. Cereal Sci.35, 65-78.
17. Zhou, Z., K. Robards K., Helliwell S., Blanchard C.andBaxterb G. 2003. Rice ageing.Effect of changes in protein on starch behavior. J. Starch-Starke. 55, 162-169.
18. Zhou, Z., Robards K., Helliwell S. and Blanchard C. 2001.Effect of rice storage on pasting properties ofrice flour. Food Res Int. 36, 625-634.

PAK. J. FOOD SCI., 22(3), 2012: 133-142
ISSN: 2226-5899

Evaluation of traceability systems in fish supply chains: A case study of Tanzania

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ABSTRACT

The European General Food Law, EC 178/2002 requires each stage in the supply chain to have access in its upstream and downstream trading partners. The regulation seeks to ensure that at each stage of food production, processing and movement through the supply chain steps are taken to maintain safety of the products intended for human consumption, at its highest quality. While the literature recognises the importance of food processing companies to have efficient traceability systems, there has been shortage of actual involvement of researchers in assessing the actual execution and performance of traceability systems in food processing companies, especially in developing countries. Using a qualitative approach, this study evaluates the performance of traceability systems in Tanzanian context using a case study of four fish processing companies. It explores how fish processing companies under given contextual situations (e.g. product complexity, production process complexity, supply chain complexity and organisation complexity) design and execute their traceability systems. The findings showed that despite high degree of complexity of contextual situations, all companies used paper based traceability system with minimum computer applications. Paper based traceability system is associated with several limitations, and may lead to poor performance given higher level of complexities of contextual situations.

Key words: Traceability, contextual factors; fish processing, Tanzania

1. Introduction

Over the past decades, quality assurance (QA) has become fundamental to food safety policy in the food industry. Much focus has been on integral quality management systems. These systems cover all stages in the food supply chain including training, research and development (Beulens *et al.* 2003). The need for QA systems is now clear due to several crises like BSE, dioxin contamination, foot and mouth disease that occurred in the last decade. Consumers and government requirements are basic driving forces beyond QA systems to guarantee food safety that comply with consumers' (customer) demands. In anticipation to this situation various national and international acknowledged certification standards like BRC, SQF, Global G.A.P and ISO22000 have been developed (Huss, Reilly, & Ben Embarek, 2000; Neeliah & Goburdhun, 2007; Ropkins & Beck, 2003). Most of these QA systems consist of quality management (e.g. ISO 9001) and food safety aspects (e.g. HACCP principles). Systems that are linked to quality assurance such as traceability and Ecolabeling, have received much attention in the food industry and agribusiness (FSA, 2002; Moe, 1998; Tall, 2001; Trienekens and Van der Vorst, 2006). Food legislation like European General Food law (EC 178/2002) demands traceability systems to be developed in all food sectors. Moreover, consumers would like to know how and where the products they purchase/consume are produced, processed, stored and transported (Van der Vorst, 2006). The European general food law seeks to ensure that at each stage of food production, processing and movement along the supply chain steps are taken to maintain safety of the products intended for human consumption, at its highest quality (EC 178/2002). However, the ability to consistently trace consignments of food through the supply chain is currently inadequate. Traceability systems have been developed at company level, however, these systems provide limited traceability, and they are fragmented, uncoordinated and inconsistent in approach (Tracefish, 2001). Van der Vorst (2003) argued that the basic idea of tracking and tracing is the possibility to determine where a certain item is located and to trace the history of that item. On the basis of that information, it should also be possible to determine the source of any problem of an item, and it should be possible to find out where the other items with the same problem are located in the supply chain. In many literatures, the concept of traceability is often used as synonym to tracking and tracing (Wilson and Clarke, 1998; Van Twillert, 1999; Van Dorp, 2002), and is used interchangeably in many studies. *Tracking* refers to the determination of the on-going location of items during their way through the supply chain (figure I). *Tracing* aims at defining the composition and the treatments an item received during the various stages in the production life cycle. Chain upstream (backward) tracing aims at determining the history of items and is used to determine the source of a problem of a defective item. The chain downstream (forward) tracing, aims at the determination of the

location of items that were produced using for example a contaminated batch of raw materials.

Between 1997 and 1999 the East African Countries (i.e. Tanzania, Kenya and Uganda) experienced two export bans of fish and fishery products from Lake Victoria due to outbreak of cholera and pesticide contamination. The European Union (EU) requested these countries to demonstrate beyond any doubt that fish from Lake Victoria did not contain pesticides residues and microbiological contamination above tolerable levels (FAO/WHO, 2002). Unfortunately, the countries failed (at that time) to respond immediately to EU request due to lack of appropriate traceability systems in place. Timely response to emerging or suspected hazards is very important if the extents of dangers as well as devastating economic losses are to be minimized. Availability of effective traceability systems would have greatly minimized colossal economic losses incurred as a result of the ban (FAO/WHO, 2002). Therefore, the aim of this paper is to analyse the contextual situation and evaluate performance of traceability systems in fish processing companies in Tanzania and propose measures for improvement.

2.0. Methodology

2.1. Characteristics of the companies

To evaluate performance of traceability systems in fish processing companies, four companies were analysed. All companies have EU approval number and their major export markets are the EU, Japan and the Middle East. Also, they have validated HACCP, GMP and traceability systems; and are regulated by the Ministry of Livestock and Fisheries Development, which is the competent authority in Tanzania. Company 'A' is a medium sized company with 120 employees, 277 suppliers and deals with sea fish processing. Company 'B' is large sized company with 600 employees, 25 suppliers and specialises in Nile perch processing. Company 'C' is also large sized company with 500 employees, 49 suppliers and specialises in Nile perch processing. Company 'D' is a large sized company with 500 employees, 12 suppliers and specialises in Nile perch processing.

2.2. Evaluation of performance of traceability systems

The evaluation consisted of two parts. Part I involved analysis of traceability system with respect to contextual factors, whereas part II analysed traceability system with respect to traceability information requirements in fish supply chain (Table 1). Contextual factors are described as the environment in which an organization operates that affect the level of performance of traceability system. Contextual factors include product complexity, production process complexity, supply chain complexity and organisation complexity/characteristics (Kousta, 2006; Luning et al., 2009; Mgonja et al., in press; Van der Spiegel, 2004). High levels of complexity of the contextual factors are assumed to put high requirements/demands on the performance of the traceability system. High requirement/demands in traceability system refers to requiring more information collection points, more detailed information, more data processing, collection of more samples, collection of samples at a higher level e.g. at ingredient level. However, organisation complexity/characteristic is considered to be somehow different from the rest of the contextual factors. Organisation complexity or characteristics contribute either positively or negatively on the design and execution of the T&T system, but does not put high/low demands. For instance high level of employee involvement (as an indicator of organisation complexity) does not put a higher requirement/demand on the T & T system but will result into proper design and execution of the traceability system.

In order to obtain an indication of the traceability system performance we selected indicators to judge how traceability systems have been appreciated and discussed by various experts in literature. The assumption behind the performance of traceability system is that food processors which evaluate performance of their traceability systems in a more structured way and according to very specific criteria will have a better insight in their situation and stand a better chance of preventing food safety problems from occurring in their companies (Jacxsens et al., 2010; Mgonja, 2007)

For each contextual factor several indicators were derived from literature (Table 1). The indicators measure the complexity of contextual factors. Typical for a performance indicator is that it includes a certain judgment (is it high or moderate or low). An indicator can provide information about or can be indicative for an overall situation (Luning et al., 2008). For each indicator three description levels were derived, low level (1), medium level (2) and high level (3). Low level refers to e.g. slight loss of flavour, not likely to occur, 1-2 species, 1-5 processing steps/lines, less than 20% supplied fish is from wild sources, ad-hoc exchange of information, no T& T system stated and reviewed, employee not involved. Medium level corresponds to e.g. unpleasant smell, likely to occur, 2-10 species, 6-10 processing steps/lines, 20-50% supplied from wild sources, regular exchange of information, T& T system stated but not reviewed, employee partly involved etc. High level refers to e.g. putrid smell, high likely to occur, more than 10 species, more than 10 processing steps/lines, more than 50% supplied fish come from wild sources, joint planning and regular exchange of information, T& T system clearly stated and reviewed, employees completely involved etc.

Table 1: Grid to Assess Contextual Factors

Product Complexity				
Indicator	Assumed mechanism	Low level	Medium level	High level
Spoilage rate of fish. (Dalgaard *et al.*, (1994), FDA (2001), FAO (1997), and Gupta and Misra (1997))	When processing fish with high spoilage rate you need to collect more information to be able to judge the safety level of fish. Thus, fish with high spoilage rate put higher demand on the design and execution of T&T system	Slight loss of natural flavour and odour when fish stored in ice for > 11 days.	Unpleasant smell when fish stored in ice for 7-10 days	Putrid smell and regarded not fit for consumption when fish kept in ice for 0-6 days
Risk level of RM/Product for safety (Based on FSA (2002), NACMCF (1998), Kousta (2006) and Van der Vorst (2004))	If the risk level of the product is high, then detailed information is required to judge safety level of the product, which put more requirements /demands on the design and execution of the T&T system.	The incident is not likely to occur once in 10 years and once it occurs, it is simply about a product being out of specification	When there is a possibility of a repeated incident i.e. once in a year and which may result to customer ill health	There is a chance of a repeated incident i.e. several times per year and which result to customer fatality
Degree of diversity of raw material with potential hazards. Based on FDA (2001) and Luten e*t al*., (2003)	Different species e.g. of fish have different Hazards. The more varied species processed the more detailed information needed to be collected. This situation puts higher requirements on T&T system	Only 1 or 2 fish species with potential hazards are processed throughout the chain	Between 2 and 10 fish species with potential hazards are processed	More than 10 species of fish with potential hazards are processed throughout the chain
Production Process Complexity				
Indicator	Assumed mechanism	Low level	Medium level	High level
Number of processing steps/lines Based on Moe, 1998; Vernède et al., 2003; Golan et al., 2005	Many production steps/lines means more points for data collection need to be included in the T&T system. This puts higher demand on the design and execution of T&T system	Between 1and 5 processing steps/lines	Between 6 and 10 processing steps/lines	More than 10 processing steps/lines
Production process structure (convergence and divergence process) Based on (Moe, 1998; Vernède *et al*., 2003; Golan *et al*., 2005 and FDA, 2001	Diverging and converging product streams make it difficult to follow the different raw materials that go into the product and all the end products. This puts high demands on the design and execution of the T&T system	Divergence/ Convergence process occurs within the company	Divergence /Convergence process occurs outside the company	Divergence/ Convergence process occurs inside and outside the company
Sources of raw materials (RM) supply. (Based on Trienekens and Van der Vorst 2006)	Having RM from wild sources (e.g. Ocean, lake) you need to do many analyses so as to judge the safety level of the RM. This situation put a higher demand on the design and execution of the T&T system	Less than 20% of raw materials are supplied from wild sources	20 - 50% of the raw materials are supplied from wild sources	More than 50% of the raw materials are supplied from wild sources
Supply Chain Complexity				
Indicator	Assumed mechanism	Low level	Medium level	High level
Degree of diversity of the chain actors (Based on Lazzarini et al., (2001), Van der Vorst (2006))	Having many actors in the chain is associated with receiving and sending more information than when there are only few actors. This situation puts a high demand on the design and execution of the T&T system.	Supply chain consists of one RM supplier, one fish processor and one buyer	Supply chain consists of multiple RM suppliers, one fish processor and one buyer or its vice versa	Supply chain consists of multiple RM suppliers, one fish processors and multiple buyers
Level of chain partnership (Based on Spekman et al., (1998)	High level of chain partnership is associated with high level of chain collaboration and sharing of all	Partners exchange bits of information (e.g. product	Partners exchange product and	Partners carry out joint planning of all activities and

and ECR Europe (2004))	business information on regular basis, which contribute positively on the T&T system design and execution and hence T&T system performance.	information, quantity, price etc.) only upon request	process information on a regular basis e.g. prices, quantity, production method etc.	exchange all information about product, process and customers on regular bases.
Organisation Complexity/characteristics				
Indicator	Assumed mechanism	Low level	Medium level	High level
Degree of employees involvement Ivancevich (1994), and Luning et al., (2002))	Early inclusion of workers in designing T&T system will lead to a better understanding of its purpose and importance. This may contribute to a more positive attitude and a more desirable intention to execute T&T system at a high level.	Employees are just informed and instructed about how to work with T&T system during execution	Employees suggestions and opinions are taken into account during designing stage	Employees are completely involved in T&T system from the moment of conceptualization, throughout the execution process
Working conditions of employees (Based on Luning *et al.*, (2002) and US Bureau of labour statistics, 2006)	Good working conditions such as good ventilation, good smell and provision of feedback information is highly motivating and positively contribute to the design and execution of the T & T system	Poor ventilation, bad smell and no feedback information	Good ventilation but no provision of feedback information	Good ventilation, good smell and provision of feedback information
Top management commitment (Based on ISO 9001:2000)	Management commitment and support is essential for T&T system. High commitment of the top management is associated with a clear policy about the T&T system design and execution, clear statement regarding the T&T system reviews and personnel responsible for the implementation of the T&T system at the managerial level	T&T system is not stated in the organisations' policy, there is no T&T system reviews and is not stated who is responsible for the implementation of the T&T system	T&T system is stated in the organisations' policy but there is no T&T system reviews and is not stated who is responsible for the implementation of the T&T system	T&T system is clearly stated in the organisations' policy, presence of T&T system reviews and it is clearly stated who is responsible for the T&T system implementation
Rate of temporary workers (Based on Foote, 2004)	Temporary workers lack; motivation, commitment, proper training, proper working skills and work experience. Large number of temporary workers will negatively contribute on the T&T system	Less than 30% of all employees are temporary employees	Between 30 and 60% of all employees are temporary employees	More than 60% of all employees are temporary employees
Traceability Information requirements				
Indicator	Assumed mechanism	Low level	Medium level	High level
Level of T & T registrations (Based on Perez *et al*., 2003, Tracefish (2001) and Regulation 2065/2001 of EU)	More extended/ comprehensive registration of traceability information contributes positively to a higher level of T&T system design and execution because it enables a more precise tracking and tracing of a problem and therefore contributes positively on the overall performance of the T&T system	Only information about method of processing, quantity, price, handling method and time/temperature	Only information about, packaging , time/temperature , appearance, expiry dates, landing dates, fishing gear and landing date	All information as required by regulation

Three indicators were developed for product complexity (i.e. spoilage rate of fish, risk level of fish product, and diversity of fish species); and four indicators were formulated for production process complexity (i.e. number of processing lines, number of processing steps, nature of production process structure, and sources of raw material supply). Also, two indicators for supply chain complexity (i.e. level of supply chain partnership and diversity of chain actors), and four indicators for organisation complexity/characteristics (i.e. employees involvement, working conditions of employees, rate of temporary workers and extent of top management commitment) were derived.

Traceability information requirements refers to information that is required by law to be registered and made available to competent authority any time whenever need arises (Perez *et al*., 2003; Regulation 2065/2001 of EU; Tracefish, 2001). Traceability information requirements is mainly composed by one indicator, namely T&T system registrations. It is assumed that the extent of implementation of the traceability system requirements can be measured by checking the degree of data registrations in the company. The evaluation of the performance of traceability systems was conducted through documents analysis, face to face interviews with QA personnel and directors (1-2 hours), and direct observations.

3. Results and Discussion

Table 2 shows the scores for the individual context indicator and the overall score for that contextual factor for all studied fish processing companies. For all companies, contextual factors had similar indicator scores, except one indicator of product complexity, (i.e. the degree of diversity of fish species). Most indicators of contextual factors scored 1 and 3, while the indicator for traceability information requirements scored 3 for all companies. Score 1 puts low demand in the T&T system, while score 3 puts higher demands on the system.

For product complexity, all analysed companies scored 1 (low score) in all indicators except one company (CI), which scored 3 (high score) with respect to degree of diversity of fish species (Table 2). More traceability information is required when dealing with many species of fish because different species of fish have different potential hazards (FDA, 2001). So a profound knowledge on specie related hazards, product reactions and synergistic effects in fish is required during processing, and designing and execution of T&T system. In general, fish industry trades globally in a vast range of fish species which have different hazards (Luten *et al*., 2003). When dealing with many species of fish with potential hazards (such as parasites, natural toxins, histamine/scombrotoxins, pesticides, antibiotics, drugs and methyl mercury), one needs to collect more samples (detailed samples) for analysis so as to precisely judge the safety level of the final product. This situation generates a large volume of data and puts higher demand/requirement on the T&T system, which may affect the overall system performance.

For production process complexity, all analysed companies had high score (score 3) in three indicators except in one indicator (number of processing lines) which had a low score (score 1). The score for the indicator "number of processing steps" was high for all companies. This indicates that the companies had more than 10 processing steps. Having many production lines/steps means also having many data generation points, which implies that more points for information collection needs to be included in the traceability system. A large volume of data put a higher demand on the design and execution of traceability system than when dealing with low volume. Moreover, generating a large volume of data is a tedious work and may be susceptible to errors (FDA, 2001), which may also affect performance of the traceability system. Therefore, a company with many processing lines/steps requires a more robust system for data capturing, storage, processing and retrieval.

Also, Table 2 denotes that the score for the indicator "production process structure" was high for all analysed companies. This implies that the companies experience both divergence and convergence processes, inside and outside of their companies. Production process structure can be a straight line, divergent and/or convergent. Diverging and converging product streams make it difficult to follow the different raw materials that go into the product and all the end products. Divergent process (where product flows diverge into a larger number of products) and convergent process (where a large number of product flows converge into a single product) are common practices during fish processing. Divergence of materials into more products generates a track of numerous different lots (e.g. brined fillet, brined loin, chilled loin, frozen log and frozen block) and therefore, more information is required to be registered in the traceability system (Moe, 1998; Golan *et al*., 2004). Vernède *et al.,* (2003) also supported the idea that registering information about the product identity is especially required before and after convergent and divergent processes. It is assumed that if divergence and/or convergence process occurs outside the company, registration of the necessary information cannot be guaranteed. On the other hand, if convergence and/or divergence process occurs within the company, registration of the necessary information can be done correctly since the process is within the control of the processor. However, convergence and divergence process

may occur both outside and inside the company and thus, put more demand on the T&T system.

Table 2: Scores of indicators representing the contextual factors and traceability information requirements for fish processing companies

Factor and Indicator	C1	C2	C3	C4	Average score
Product Complexity					
Spoilage rate of fish	1	1	1	1	1
Risk level of fish product	1	1	1	1	1
Degree of diversity of fish species	3	1	1	1	2
Production process complexity					
Number of processing steps	3	3	3	3	3
Number of processing lines	1	1	1	1	1
Production process structure	3	3	3	3	3
Sources of raw material supply	3	3	3	3	3
Fish Supply Chain Complexity					
Degree of diversity of chain actors	3	3	3	3	3
Level of chain partnership	3	3	3	3	3
Organisation Characteristics/Complexity					
Degree of employee involvement	1	1	1	1	1
Working conditions of employees	1	1	1	1	1
Rate of temporary workers	3	3	3	3	3
Level of top management commitment	2	2	2	2	2
Traceability System Requirements					
Level of registration of traceability information	3	3	3	3	3

C1=Company 1, C2=Company 2, C3=Company 3 and C4=Company 4

Table 2 indicates that the score for the indicator "Sources of raw material supply" was also found to be high in all analysed companies. This shows that 100% of the raw materials are supplied from wild sources (ocean and Lake). Wild sources of raw material supply make traceability system more complicated since the company will be obliged to do more tests (microbiological and chemical) to adequately judge the safety level of the raw material and the final product. In other words, if the company obtains its fish from a specific farm, which operates with clear specifications, then hazards can be well established/ predicted and the safety level can be determined. On the other hand, if the company obtains its fish from wild sources (ocean/lakes), then it is difficult to establish/ predict all the hazards. Thus, the raw material may have a higher chance of being associated with unknown hazards that would require more analysis. According to FSA (2005), fishes originating from wild sources have high chance of associated with higher level of toxicological contaminants (such as methyl mercury and dioxins and dioxins like PCBs) and thus, present a highest safety risk. Raw materials supplied from wild sources therefore, put a higher demand on the design and execution of the traceability system than farmed fish.

With regards to fish supply chain complexity, all analysed companies had high score (score 3) in both indicators; degree of diversity of chain actors, and level of chain partnership (Table 2). High degree of diversity of chain actors implies that the processing companies have multiple suppliers and buyers. When the firm has many chain actors (Lazzarini *et al.,* 2001), then more information exchange is required. This puts more demand in the traceability system as more information would be required for exchange than when dealing with a few actors. Similarly, high level of chain partnership indicates that partners (e.g. fish suppliers, processors and buyers) carry out joint planning of all activities and exchange all information about products, processes and customers on regular basis. It is assumed that as the level of chain partnership increases partners dedicate more resources to sustain and further the goals of the supply chain; as a result information is more easily exchanged (Lazzarini *et al.,* 2001). Also, when the level of chain partnership is low, there is less information to be transferred since partners exchange bits of essential information only upon request. When the level of chain partnership is high, there is more information to be transferred because partners carry out joint planning of all activities and exchange all information on regular basis. Therefore, high level of chain partnership is associated with more/detailed information sharing, and thus positively contributes to the design, and execution of the traceability system

With regards to organization characteristics/complexity, all companies had a low score in degree of employees' involvement and working conditions; and high score in rate of temporary workers (Table 2). High score in rate of temporary workers means that temporary employees in these companies are more than 60%. Temporary employees are in a constant state of employment flux, and therefore, high turnover. This is due to the fact that they are neither guaranteed consistent employment nor assured of a solid start or finish date for their assignments. Temporary workers lack motivation and commitment, proper training, skills and experience (Foote, 2004). If the company is characterised by a large number of temporary workers, it is likely that many quality assurance activities including T&T will not be carried out perfectly. This situation may affect performance of the T&T system.

For the traceability information requirements, all companies had a high score in the level of registration of traceability information (Table 2). This is probably due to the fact that these companies are all regulated by the competent authority of Tanzania and are required to meet the EU regulation 2065/2001 on traceability information requirements. According to EU Regulation 2065/2001, the following traceability information should be made available to the competent authority: supplier name, origin of fish, boat registration number, quantity supplied, common name, scientific name, company name, company address, processing date, production method, expiry date, carton number, carton weight, code or grade, storage temperature, destination, source of packaging material and FAO fishing zone. Also, all suppliers to the EU should indicate their respective EU approval number. High level of registration indicates that all necessary information required for traceability purposes as demanded by the law was correctly registered by the companies. This enables a more precise tracking and tracing of food safety problems whenever they occur. However, it was observed that all companies used paper based registration system with limited computer application. Paper based registration system has several limitations including inadequate functionalities, time consuming, information cannot be easily retrieved, not fraud proof, and requires more space (Marshall, 2004; Petersen & Green 2005).

4. Conclusions

The evaluation of performance of traceability system in our study based on 14 indicators derived from four contextual factors (product complexity, production process complexity, and supply chain complexity and organisation characteristics/complexity) and traceability information requirements in fish supply chain. The study found that 7 indicators had high scores while 5 indicators had low score and 2 indicators had medium score. High

score implies that the fish companies have high contextual complexity situations which put high demands on the T&T system. High levels of complexity of contextual situation require a robust system to effectively capture, store, process and retrieve information. On the other hand, a low level of complexity of contextual situation does not put high demand on the traceability system since a very simple traceability system (e.g. paper based) can be sufficient to realise a good food safety output.

The study found that all analysed companies rely on paper based traceability system with limited computer applications despite of high degree of complexity of their contextual situations. Although paper based traceability system is the simplest form of recording information and tends to be at least part of most traceability systems, it can only be appropriate for simple processes (low complexities). The paper system relies on the user to formulate effective recording templates that can be used to record the vital parameters associated with the product, so it is prone to human errors. The paper system can appear to be the most straightforward and least cost option for a small operation, however the operator must consider the time needed to record and maintain paper records and the ability to cross reference through records if a problem occurs (Marshall, 2004). Nevertheless, the disadvantages of paper based T&T system outweighs the advantages as the level of complexity of the contextual factors increases. For this case, a more advanced T&T system would be more appropriate in these companies if higher level of traceability system and hence food safety assurance is to be achieved.

Performance diagnosis can be a useful and cheap tool to obtain a quick impression on how fast and correctly companies can trace a food safety problem in their companies. The assumption behind the traceability system performance diagnosis is that processing companies that evaluate performance of their food safety management system in a more structured way and according to specific criteria will have a better insight in their actual performance and food safety problems can be more systematically detected (Jacxsens *et al*,. 2010). The self-assessment provides insight in the strong and weak points of the current system and supports a food business in identifying what/how to improve (Sampers et al., 2010). Besides the usefulness of this tool for an individual food processor, the traceability performance diagnosis tool can also be applied on the level of governments (e.g. Competent Authorities) to benchmark performance of implemented traceability systems in various other sectors in the country.

Acknowledgement

This study was partly supported by Netherlands Fellowship Programmes (NFP)

REFERENCES

1. Beulens A.J.M, Broens D.F, Folstar P and Hofstede G.J (2003). Food Safety and Transparency in Food Chain and Networks Relationships and Challenges. Wageningen University. The Netherlands.
2. Dalgaard, P. (1995). Qualitative and quantitative characterization of spoilage bacteria from packed fish. *Int. J. Food Microbiology. 26 (3), pp*.319-333.
3. ECR Europe (2004). Efficient Consumer Réponse. Using traceability in the supply chain to meet consumer safety expectations. [March 2004] http:// www.ecrnet.org.
4. FAO. (1997). The State of Food and Agriculture. Rome: Italy.
5. FAO/WHO (2002). Reduction of foodborne hazards, including microbiological and others, with emphasis on emerging hazards. Global Forum of Food Safety Regulators Marrakech, Morocco, 28-30 January, 2002.
6. FDA (2001). Food & Drug Administration. Centre for Food Safety & Applied Nutrition. Fish and Fishery Products Hazards and Controls Guide 3rd Edition (http://www.cfsan.fda.gov/~comm/haccp4.html).
7. Food Standards Agency (FSA, 2002). Traceability in the food chain. A preliminary study Food Chain Strategy Division, Food Standard Agency.[March 2002] http://www.food.gov.uk/multimedia/pdfs/traceabilityinthefoodchain.pdf
8. Food Standard Agency (FSA, 2005) EFSA advice on the safety and nutritional contribution of wild and farmed fish. Parma, Italy. [October, 2005]. http://www. Efsa.eu.int.
9. Foote, D.A. (2004). Temporary workers, managing the problem of unscheduled turnovers. *Emerald management* Vol. 42 No. 8 pp. 963-973.
10. Golan E., Krissoff B., Kuchler F., Nelson K. & Price G. (2004). – Traceability in the US food supply: economic theory and industry studies. Agricultural Economic Report No. AER830, Economic Research Service of the United States Department of Agriculture.
11. Gupta, S. Misra TN. 1997. Manganese phthalocyanine for the detection of fish freshness byits trimethylamine emission.Sensors and Actuators, B: Chemical B41(1-3): 199-202
12. Huss, H. H., Reilly, A., & Ben Embarek, P. K. (2000). Prevention and control of hazards in seafood. *Food Control*, 11(2), 149-156.
13. ISO 22000, Food safety management systems: Requirements for any organization in the food chain. BS EN ISO 22000:2005 [2005-09-01]. http://www.cmcq.com.cn/download/ISO22000.pdf
14. ISO 9001: 2000. Quality Management Systems Design
15. Ivancevich, J.M., Lorenz, P. and Skinner, S. (1994). Management, Quality and

Competitiveness. Richard D. Irwin, Boston. MA, 1994, pp40-67.

16. Jacxsens, L., Uyttendaele, M., Devlieghere, F., Rovira, J., Oses Gomez, S., and Luning P.A. (2010). Food safety performance indicators to benchmark food safety output of food safety management systems. *International Journal of Food Microbiology* 141:S180–S187
17. Kousta Maria (2006). Food Quality Management master's thesis. Wageningen University, Netherlands.
18. Lazzarini S.G., Chaddad F.R and M.L. Cook M.L (2001). Integrating supply chains and network analyses, the study of netchains. In: J. on Chain and Network Science, Vol. 1.
19. Luning. P. A, Marcelis W. J and Jongen, W.M.F (2002). Food Quality Management, a techno-managerial approach, Wageningen University, The Netherlands.
20. Luning, P.A., Bango, L., Kussaga, J., Rovira, J., Marcelis, W.J., 2008. Comprehensive analysis and differentiated assessment of food safety control systems: a diagnostic instrument. *Trends in Food Science & Technology* 19, 522–534.
21. Luning, P.A., Marcelis W.J. Rovira., Van der Spiegel, M,. Uyttendaeled, M. and Jacxsensd, L. (2009). Systematic assessment of core assurance activities in a company specific food safety management system. Trends in Food Science & Technology 20: 300-312.
22. Luten. J. B, Oehlenschlager J, Olafsdottir G (2003). Quality of Fish from catch to consumer. Labelling, Monitoring and traceability. Wageningen, the Netherlands.
23. Marshall Peter (2004). Traceability module, AquaTT Student Workshop, Independent Food Quality Certification (I: FQC). (www.aquatt.ie)
24. Mgonja, J.T. (2007). Understanding Technological and Managerial Factors contributing on the Performance of Traceability Systems in the Fish Supply Chain, the case study of Tanzania. Master thesis. Food Quality Management. Wageningen University. The Netherlands.
25. Mgonja, J. T., Luning, P.A and Van der Vorst J.G.A.J. (in press). Conceptual Framework to Diagnose Performance of Implemented Traceability System in Food Processing Companies. Food Control Journal
26. Moe, T. (1998). Perspectives on traceability in food manufacture. Trends in Food Science and Technology.9:211-214
27. NACMCF (1998). National Advisory Committee on Microbiological Criteria for Foods, US Food Safety and Inspection Service. Principles of risk assessment for illness caused by foodborne biological agents. J Food Protect. 61(8):1071-1074
28. Neeliah, S. A., & Goburdhun, D. (2007). National food control systems: a review. *Food Reviews International*, 23(1), 35-51.
29. Petersen, A. and Green, D. (2005). Seafood Traceability: A practical guide for the U.S. industry. North Carolina State University [20/05/2007]. http://www.ncsu.edu/foodscience/seafoodlab/publications/SeafoodTraceabilityBook.pdf
30. Pérez-Villarreal Begoña, Isabelle Letellier, Henri Loréal and Monique Etienne (2003). Fish quality labelling systems in Southern Europe In: "Quality of Fish from Catch to Consumer: Labelling, Monitoring and Traceability". Edited by J.B. Luten, J. Oehlenschläger and G. Ólafsdóttir. Wageningen Academic Publishers.
31. Ropkins, K., & Beck, A. J. (2003). Using HACCP to control organic hazards in food wholesale, distribution, storage and retail. *Trends in Food Science & Technology*, 14(9), 374-389.
32. Sampers, I., Jacxsens, L., Luning, P.A., Marcelis, M.J., Dumoulin, A., Uyttendaele, M. (2010. Performance of food safety management systems in poultry meat preparation processing plants and relation to Campylobacter spp. contamination. Journal of Food Protection 73 (8): 1447-57.
33. Spekman E. R, Kamauff Jr, J.W and Myhr N. (1998). An empirical investigation into supply chain management. A perspective on partnerships. *International journal of physical distribution & logistics Management*, Vol.28 No.8.
34. Tall, A. (2001). Traceability procedures based on FDA and CFIA regulations – an understanding. *Infofish International* 5:49-51.
35. Tracefish (2001). European Commission Concerted Action Project QLK1-2000-00164. Traceability of Fish Products. *Second Draft Information Standard for the Captured Fish Distribution Chains.* [05.12.2001] http://www. Tracefish.org.
36. Trienekens J.H. and Van der Vorst J.G.A.J. (2006). Traceability in Food Supply Chains, in: Safety in Agri-Food Chains, (Eds) Luning, P.A., DeVlieghere, F., Verhé, R., Wageningen Academic Publishers, p. 439-470.
37. U.S. Bureau of labour statistics, Department of labour, Career to industries, 2006-07 Editions, [16.06.2007) http://WWW.bls.gov/oco/cg/cgs011.htm.
38. Van der Spiegel M. (2004). Measuring effectiveness of food quality management. PhD thesis Wageningen University, Wageningen, The Netherlands.
39. Van der Vorst, J.G.A.J. (2004): Performance levels in food traceability and the impact on chain design: results of an international benchmark study, In: H.J. Bermmers, -S.W.F. Omta, – J. H. Trienekens and E.F.M. Wubben (eds), Dinamics in chains and networks, Wageningen Academic Publishers, Wageningen, the Netherlands.
40. Van der Vorst, J. (2006). Product traceability in food-supply chains. *Accreditation and Quality Assurance: Journal for Quality, Comparability and Reliability in Chemical Measurement.* 11(1-2): 33-37.
41. Van Dorp, K. J., (2002). "Tracking and tracing: a structure for development and contemporary

practices", *Logistics Information Management*, Vol. 15, No. 1, pp.24–33.

42. Van Twillert, J. (1999). "Tracking and Tracing in Semi-process Industries," Development of a Generalised Concept for the Baan Series, Wageningen Agricultural University (WAU), Wageningen.
43. Vernede, R., Verdenius, F., and Broeze, J., (2003): "Traceability in food Processing Chains: State of the Art and Future Developments". Wageningen University, Agrotechnology and Food Innovations bv., KLICT Position Paper, Version 1.0.
44. Wilson, T.P. and Clarke, W.R. (1998). "Food safety and traceability in the agricultural supply chain: using the Internet to deliver traceability", *Supply Chain Management:An International Journal*, Vol. 3, No. 3, pp. 127–133.

PAK. J. FOOD SCI., 22(3), 2012: 154-160
ISSN: 2226-5899

Development and evaluation of fruit flavored soymilk

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ABSTRACT

Soymilk was prepared by soaking forenight with bicarbonate of soda (baking soda) drained, and then blanched to inactivate lipoxygenase enzyme. Beans were passed through blender and extract was squeezed through muslin cloth, adjusted TSS of the remaining milk to 12% with distilled water, and homogenized. The milk was sweetened with 5% cane sugar and divided to four equal lots. Three lots were flavored separately with most acceptable quantity (10%) of mango, banana and guava fruit pulp and one lot was kept as control. All the samples were pasteurized at 161 ^{o}F for 16 minutes, cooled and packed in 250ml sterilized glass bottles and stored in refrigerator (40-45 ^{o}F). All the samples were analyzed for pH, acidity, TSS, protein and fat and organoleptically evaluated at an interval of one week till coagulation. It was observed that pH decreased while acidity and TSS significantly ($P < 0.05$) increased in all the samples. There was no effect of storage on protein content of all samples. The fat content remained almost the same. The score for color and flavor significantly ($P < 0.05$) decreased during storage. All the samples coagulated after four week storage.

Keywords: fruit flavored soymilk, soybean, mango, banana, and guava

INTRODUCTION

Legumes represent a broad area in human foods and animal feeds, known to be excellent sources of protein, also rich in important vitamins and minerals. The oil seed legumes soybeans and peanuts are most frequently used as a source of vegetable oil. Soyabean (*Glycine max* L.) belongs to the family Leguminosea sub family papilionoceae and genus glycine, are the most widely grown of the grain legumes. It is one of the most important protein and oil crops in the world. Soybean is a universal food and is used as feed, fodder and, industrial crop. It is used to prepare more than four hundred different products. Its bye products are also used in manufacturing of soap, varnish, pants, lubricants, and plastics. It is used in food industry in the manufacturing of flour, oil, margarine, biscuits, candy, milk, meat, cheese, lecithin and many other food products. Nutritionally it is much superior to all conventional pulses like grain, lentil, mong, mash, pigeon pea, cowpea and various beans.

Soybean is low in carbohydrates, thus useful for diabetic patients as well as for weight reducing purposes. It is far superior in the supply of essential amino acid and fatty acids. It also contains almost all vitamins and more minerals as compared to other pulses. Soybean contains 37.54 to 45.60% protein, 19.40 to 27.00% crude fat, 0.17 to 0.21% Ca (Ahmed *et al.*, 1983). Soybean is reported to have a high nutritive value, being rich in both lysine and tryptophane, which are generally deficient in diets based on cereals. Soybean protein may give good blend with the protein of cereals.

Soybean products have been an integral part of modern food processing industry. Soyflours concentrates and isolates are found in many of the food products and have added variety to our food choices. Amongst the various concentrates soy products, soymilk, an intermediate product in the production of tofu is also produced commercially. In the United State, China and other Far East countries soymilk is used by individuals who are allergic to the protein of cow's milk, or who are lactose intolerant. Soymilk is popular amongst vegetarian who wish to totally avoid animal products.

Soymilk may be used in place of cow's milk in a number of recipes. Soymilk is sold around the world in many cases; it is flavored or sweetened to suit local preferences. Added ingredients change the nutrient content depending on the flavoring, or sweetener used. Soymilk has been known in China for centuries. It is mostly produced in the home and sometimes on a small scale. Recently considerable progress in the large scale production of soy beverage has been reported and quote of few soy beverage are marketed commercially in Hong Kong, Japan, Taiwan, Thailand, Singaporean Malaysia. A 100 gram portion (about 1/2cup) of soymilk provides 3.20 gram of protein, comparing favorably with cow's milk which contains 3.29 gram. On the other hand soymilk contains less Calcium, Phosphorus and sodium. Soymilk, however contain more iron, magnesium and potassium than whole cow's milk.

Soymilk has been used in China for centuries in much the same ways that cow milk has been used in the west. Soymilk could serve as practical and inexpensive source of high quality essential nutrients both for infants, growing children and for adults of all ages. Therefore, this project has been taken to bridge the nutritional gap in developing countries which in the direction of developing nutritious beverages based on inexpensive vegetable, protein, as animal milk is in short supply and is rather expensive in most of the countries. Soybean has a

particular beany flavor which is not like by most consumers. To overcome this, the milk was sweetened with sugar and flavored with fruit flavors. It is hope that this project will help in overcoming the deficiencies of milk and will also help the people interested in preparation of milk beverages.

MATERIALS AND METHODS

Soybean seed

The research work was conducted in the department of Food Science & Technology, NWFP Agricultural University, Peshawar. Certified seeds of soybean variety Swat-84 were obtained from Agronomy departmentof NWFP Agricultural University Peshawar and were used for the preparation of soymilk. Soybean seeds were cleaned by removing all the stones, pebbles, and unwanted material. Then seeds were washed twice with clean water thoroughly.

Soaking of soybean

One Kg of soybean was soaked in three kg of water i.e. (1:3) containing 50 gram of sodium bicarbonate (baking soda) for eight hours. After soaking the soybean seeds were blanched for 25 minutes in boiled water containing baking soda in order to destroy lipoxygenase enzyme. The blanched seeds were drained and ground in the blender with small amount of warm water to form a paste. The paste was diluted to 10-12 total solids with water. The soymilk so prepared was heated to 180 degree F for 12 minutes. After heating the soymilk was homogenized (Escuta and Benzon, 1979).

Preparation of flavored soymilk

The soymilk samples were flavored with different amounts of flavors i.e. mango, guava and banana. One sample remained unflavored i.e. controlled. The samples were homogenized, sweetened with 5% cane sugar, filled in to 250ml sterilized glass bottles, pasteurized at 161F for 16 minutes and cooled. The flavored soymilk samples in case of each flavor were subjectively analyzed by a panel of 10 judges to find out the most acceptable flavored soymilk drink sample. After finding of the most acceptable drink samples in case of each flavor, the samples were prepared, homogenized, filled in glass bottles, pasteurized at 161 F for 16 minutes cooled and stored in refrigerator for further study.

Organoleptic evaluation

For organoleptic evaluation was conducted out by using 9 point Hedonic scale as describe for Larmond (1977) starting from extremely dislike to extremely like.

Analytical work

The freshly prepared unflavored as well as flavored soymilk drinks were analyzed for pH, total soluble solids, titratable acidity, crude protein and crude fats during storage to see the effect of storage on its chemical composition.

pH

pH was determined with the help of pH meter. The pH meter was standardized by using buffer solutions before measuring the pH of the sample. Electrode was immersed in sample taken in a beaker, switched to pH and reading was taken.

Total soluble solids

The total soluble solids were determined by the standard method of A.O.A.C (1984) using hand refractometer at room temperature.

Total titratable acidity

Total acidity was determined by standard method of A.O.A.C (1984).

Crude protein

Protein percentage of the soymilk was estimated by the kjeldhal method as recommended by A.O.A.C (1984).

Crude fat

Fat percentage of the soymilk was determined by the standard method of Babcock as recommended by A.O.A.C (1984).

RESULTS AND DISCUSSION

Preparation of Soymilk

Soymilk was prepared (Escueta and Banzon, 1979) by traditional method, sweetened with 5% cane sugar. Different amount of flavors of different fruits, such as mango, guava and banana were added to soymilk samples @ 7, 9 and 10%. One of the soymilk samples was remained controlled i.e. unflavored.

Four samples in different concentrations in case of each flavor were presented to 10 judges to find out the most acceptable drink in case of each flavor. 7 ml/100 ml was taken as a base line for all the three samples. However, sample with 10% of fruits pulp was accepted by most of the judges. After finding the most acceptable drink samples amongst the four soymilk samples in case of each flavor was prepared in large quantity and stored in refrigerator (40-45 ^{o} F). All the samples were analyzed for various chemical parameters such as pH, acidity, TSS, protein and fat content and organoleptically evaluated for color and flavor at fresh and after one week for a total period of 28 days at 40-45 ^{o}F. The data regarding each parameter are presented in tables, statistically analyzed and discussed as under:

pH

The most acceptable soymilk samples flavored with mango, guava, banana and unflavored soymilk were analyzed for pH. After preparation the pH value for

guava, mango, banana and control soymilk samples was 5.8, 5.8, 6.8 and 7.0 respectively (Table 1). During storage period there was a slight and continuous decrease in pH values in all fruit flavored soymilk samples. The mean values were 5.38, 5.4, 6.36 and 6.6 for guava, mango, banana flavored and unflavored soymilk samples respectively. The statistical analysis showed that the pH values significantly ($P \leq 0.05$) decreased in all samples during storage.

These results are in agreement with Gold *et al.* (1946) who observed that titratable acidity increases and pH decreases in milk during storage. The findings regarding pH are also in agreement with the findings of Nelson *et al.* (1978) who found the average pH of whole soymilk was 7.2. These results are also in agreement with Escueta and Banzon (1979) who examined 5 samples of soymilk prepared by five different processing methods and found that their mean pH was 6.96. These results are in agreement with the finding s of Munir *et al.* (1985) that the pH of pasteurized and sterilized soymilk was 7. The author also pointed out that pH decreased with storage period. Our results are also in agreement with those of Nsofor *et al.* (1993) who investigated that pH of soymilk decreased on coagulation.

Titratable Acidity

The most acceptable soymilk samples flavored with mango, guava, banana and unflavored soymilk were analyzed for % titratable acidity. Titratable acidity on initial stage ranged between 0.386 to 0.229 (Table 2). A gradual increase was noticed during the whole storage period. The mean values for guava flavored soymilk sample was 0.392, while for mango flavored soymilk the mean value was 0.406 and 0.286 and 0.235 for banana and controlled soymilk samples respectively (Table 2). Statistical analysis showed that titratable acidity significantly ($P \leq 0.05$) increased in all samples during storage.

These results are in agreement with Gould *et al.* (1946) who investigated that titratable acidity increases in milk during storage. These results are in agreement with the findings of Munir *et al.* (1985) who found that titratable acidity increases during storage. In another study Webb *et al* (1974) reported that the acidity of milk increases with temperature, partially as a result of changes in the buffer capacity of the milk salts and the expulsion of carbon-dioxide on heating. This increase 8in acidity might be due to the thermal decomposition of the lactose to organic acids. These results are also in agreement with Gorner *et al.* (1977) who observed a gradual increase in the titratable acidity at an irregular rate and attributed it to changes in milk proteins. These results are also in agreement with Wilkowke (1954) who while studying the relationship between acidity and pH during lactic acid fermentation, found that acidity increased by increase in solids accompanied by a slight decrease in pH. Our results are also in agreement with Hussain (1986) observing that at low temperature the increase in acidity was more pronounced.

Total Soluble Solids

The most acceptable soymilk samples flavored with mango, guava, banana and unflavored soymilk were analyzed for total soluble solids. Results showed that the TSS of all samples increased during storage. The TSS of guava flavored soymilk increased from 11 to 13. Same increase in TSS was observed in mango flavored and banana flavored soymilk samples, while TSS of controlled soymilk increased from 10 to 12. The mean value for guava flavored soymilk sample was 12.40, while for mango flavored soymilk the mean value was 11.80 and 12.2 and 11.0 for banana flavored soymilk and controlled soymilk samples respectively. The % increase was observed in controlled soymilk i.e 20 % followed by the three fruit flavored soymilk samples i.e. 18% during storage (Table 3). Statistical analysis showed that TSS significantly ($P \leq 0.05$) increased in all samples during storage .

There results are in agreement with Rehman (1989) finding out that the initial mean values for the banana based milk drinks ranged from 14.97 to 15.20 ^{o}Brix. Al-Haq (1988) inferred that the rise in the total soluble solids of the pasteurized mango fruit-flavored milk-based drink could be due to the formation of pectic substances from the stabilizer Mexpectin R.S-450. In another study Kanujoso and Luh (1967) reported that the increase in T.S.S of samples may be attributed to the formation of water soluble pectin from protopectin during storage as well as hydrolysis of sucrose.

Table 1. Effect of Storage period on the pH of soymilk.

Samples	Storage interval days					Means
	0 week	Ist Week	2nd Week	3rd Week	4th Week	
Guava flavored Soymilk	5.8	5.4	4.3	5.3	5.1	5.38 C
Mango flavored Soymilk	5.8	5.5	5.3	5.2	5.2	5.4 C
Banana flavored Soymilk	6.8	6.6	6.5	6.1	5.9	6.3 B
Controlled Soymilk	7	6.9	6.5	6.3	6.3	6.6. A
Means	6.35 A	6.1 E	5.9 C	5.7 D	5.62 E	

Figures bearing the same letters are statistically not different from one another (P < 0.05).

Table 2. Effect of Storage period on the Titratable acidity of soymilk.

Samples	Storage interval days					Means
	0 week	Ist Week	2nd Week	3rd Week	4th Week	
Guava flavored Soymilk	0.386	0.386	0.39	0.39	0.41	0.392 B
Mango flavored Soymilk	0.404	0.405	0.405	0.406	0.41	0.406 A
Banana flavored Soymilk	0.27	0.28	0.28	0.29	0.31	0.286 C
Controlled Soymilk	0.229	0.229	0.23	0.24	0.25	0.235 D
Means	0.322E	0.325 D	0.326 C	0.331 B	0.345 A	

Figures bearing the same letters are statistically not different from one another (P < 0.05).

Table 3. Effect of Storage period on the Total soluble solids of soymilk samples.

Samples	Storage interval days					Means	%gain
	0 week	Ist Week	2nd Week	3rd Week	4th Week		
Guava flavored Soymilk	11	12	13	13	13	12.4 A	18
Mango flavored Soymilk	11	11	12	12	13	11.8 C	18
Banana flavored Soymilk	11	12	12	13	13	12.2 B	18
Controlled Soymilk	10	11	11	11	12	11 D	20
Means	10.7 E	11.5 D	11.7 C	12.5 B	12. 7 A		

Figures bearing the same letters are statistically not different from one another (P < 0.05).

Protein

The most acceptable soymilk samples flavored with mango, guava, banana and unflavored soymilk samples flavored with mango, guava, banana and unflavored soymilk were analyzed for protein content. Results showed that the protein content decreased in all samples during storage. The protein content of guava flavored soymilk decreased from 4.08 to 4.05. A slight decrease was noticed in all the remaining soymilk samples during storage. The mean value for guava flavored soymilk sample was 4.068, while for mango flavored and controlled soymilk samples respectively. The % losses during storage in protein content were negligible i.e. 0.73 % in guava flavored soymilk followed by banana flavored soymilk i.e. 0.49% followed by mango flavored soymilk and controlled flavored soymilk i.e. 0.24 % (Table 4). Statistical analysis showed that the effect of storage on protein content was non-significant.

These results are in agreement with Ward (1995) who found that soymilk contains 4.4 % protein. The findings of protein content of controlled soymilk is in agreement with the findings of Khaleques *et al.* (1970) who found out that soymilk contained 3.62 percent proteins.

Crude Fat

The most acceptable soymilk samples flavored with mango, guava, banana and unflavored soymilk were analyzed for fat content. Results showed that the fat content decreased during storage. The fat content on initial stage ranged between 2.4 to 2.1. The fat content of guava flavored soymilk decreased from 2.4 to 2.2. Similarly there was 2.3 to 2.2, 2.6 to 2.5 and 2.1 to 2.0 decrease of fat content in mango flavored, banana

flavored and unflavored soymilk sample was 2.280 while for mango flavored soymilk the mean value was 2.240 and 2.580 and 2.04 for banana and controlled soymilk respectively. During storage the % losses were 8.3 % in guava flavored soymilk, 4.7 % in controlled soymilk followed by mango flavored soymilk i.e. 4..3 % followed by banana flavored soymilk i.e. 3.8% (Table 5). Statistical analysis showed that storage interval had a significant ($P \leq 0.05$) effect on fat content. The fat content of the soymilk is in agreement with the findings of Altschul (1965) who found that soymilk contained 2% fat. These results are also in agreement with Ward (1995) who found that soymilk contained 2.5% fat.

Organoleptic evaluation

The samples were sensory evaluated for color and flavor during storage at an interval of one week for a total period of four weeks by a panel of ten judges experienced in organoleptic evaluation of foods. The evaluation was carried out according to 9 points Hedonic scale where 9 indicated extremely liked and one indicated extremely disliked.

Color

The most acceptable soymilk samples were organoleptically evaluated for color. Results showed that the score for color decreased during storage. The score for color of guava flavored soymilk decreased from 7.1 to 4.7. Similarly there was 6.4 to 2.7, 7.7 to 3.5 and 6.8 to 3.4 decrease in color score for mango flavored, banana flavored and unflavored soymilk samples respectively. During storage the mean value for guava flavored soymilk sample was 5.78, while for mango flavored soymilk sample. The mean value was 4.42 and 5.4 and 4.7 for banana flavored and controlled soymilk samples respectively. During storage there were 57 % losses in mango flavored soymilk followed by banana flavored soymilk 54%), controlled soymilk (50%) and guava flavored soymilk sample (33%) (Table 6). Statistical analysis showed that there was a significant (($P < 0.05$) decrease in color score during storage.

Flavor

The most acceptable soymilk samples were organoleptically evaluated for flavor. Results showed that the score for flavor decreased during storage. The score for flavor of guava flavored soymilk samples decreased from 7.0 to 3.4. Similarly there was 7.4 to 3.5, 7.1 to 3.2 and 5.5 to 2.2 decrease in flavor score for mango flavored, banana flavored and controlled soymilk samples respectively. During storage the mean value for guava flavored soymilk was 5.18, while for mango flavored soymilk samples the mean value was 5.48, and 4.94 and 3.8 for banana flavored and controlled soymilk samples respectively. During storage the % losses in flavor were high for controlled soymilk i.e. 60% followed by banana flavored (54%), mango flavored soymilk (52%) and guava flavored soymilk sample (51%) (Table 7). Statistical analysis showed that there was a significant (($P < 0.05$) decreased in flavor score during storage.

Table 4. Effect of Storage period on the protein contents of soymilk.

Samples	Storage interval days					Means	% Loss
	0 week	Ist Week	2nd Week	3rd Week	4th Week		
Guava flavored Soymilk	4.08	4.08	4.08	4.05	4.05	4.068 A	0.73
Mango flavored Soymilk	4.02	4.02	4.02	4.02	4.01	4.018 B	0.24
Banana flavored Soymilk	4.04	4.04	4.03	4.03	4.02	4.032 B	0.49
Controlled Soymilk	4.02	4	4.02	4.05	4.01	4.02 B	0.24
Means	4.047 A	4.035 A	4.037 A	4.022 A	4.022 A		

Figures bearing the same letters are statistically not different from one another ($P < 0.05$).

Table 5. Effect of Storage period on the fat contents soymilk.

Samples	Storage interval days					Means	% Loss
	0 week	Ist Week	2nd Week	3rd Week	4th Week		
Guava flavored Soymilk	2.4	2.4	2.3	2.1	2.2	2.280	8.3
Mango flavored Soymilk	2.3	2.3	2.3	2.1	2.2	2.240	4.3
Banana flavored Soymilk	2.6	2.6	2.6	2.6	2.5	2.580	3.8
Controlled Soymilk	2.1	2.0	2.1	2.0	2.0	2.040	4.7
Means	2.350	2.325	2.235	2.200	2.225		

Figures bearing the same letters are statistically not different from one another ($P < 0.05$).

Table 6. Effect of Storage on Color of Soymilk Samples

Samples	Storage interval days					Means	% Loss
	0 week	Ist Week	2nd Week	3rd Week	4th Week		
Guava flavored Soymilk	7.1	6.1	5.8	5.2	4.7	5.78 A	33
Mango flavored Soymilk	6.4	5.1	4.4	3.5	2.7	4.42 B	57
Banana flavored Soymilk	7.7	5.7	5.4	4.7	3.5	5.4 A	54
Controlled Soymilk	6.8	5.5	4.8	3.0	3.4	4.7 B	50
Means	7.0 A	5.6 B	5.1 B	4.1 C	3.57 C		

Figures bearing the same letters are statistically not different from one another ($P < 0.05$).

Table 7. Effect of Storage on Flavor of Soymilk Samples.

Samples	Storage interval days					Means	% Loss
	0 week	Ist Week	2nd Week	3rd Week	4th Week		
Guava flavored Soymilk	7.0	6.1	5.3	4.1	3.4	5.18 B	51
Mango flavored Soymilk	7.4	6.5	5.6	4.4	3.5	5.48 A	52
Banana flavored Soymilk	7.1	6.1	4.7	3.6	3.2	4.94 B	54
Controlled Soymilk	5.5	4.9	4.0	2.4	2.2	3.8 C	60
Means	6.75 A	5.9 B	4.9 C	3.6 D	3.07 E		

Figures bearing the same letters are statistically not different from one another ($P < 0.05$).

CONCLUSIONS

There is need for the encouragement from government side as well as from the private sector to introduce processed soybean products like soymilk on commercial scale. All the required necessities for manufacturing, packaging, storage and marketing should be properly provided by the concerned authorities. Efforts should be made to create awareness among the masses about the importance of processed soybean products, especially soymilk and also the inexpensive protein source. Due to the population explosion and shortage of good quality protein, efforts should be made to introduce cheapest products of high quality nutritional value like soymilk having more protein compared with cow's or buffalo's milk. Care should be taken not to add as much quantities of fruits which will result in the coagulation of drink more early than the usual shelf life of soymilk. Care should be taken not to add more acidic fruits like citruses etc. Some suggestions reveal that bleaching of dry beans can remove the undesirable beany flavor, the biggest hurdle in the acceptance of soymilk among consumers. Suitable methods should be introduce to increase its shelf life ,like addition of anticurdling powder, drying of soymilk, canning and sterilization + using tetra packs for its packaging. The residues remaining after extraction of soymilk should be treated and should be used to introduce more useful products like soymeat, a special food for diabetes patients. Treatment should be worked out to avoid the problem of settling during storage of soymilk. Research should be made to use the soymilk in the same ways that of cow's and buffalo's milk, like for cheese, yoghurt, curd production and also in bakery. Efforts should be made to develop taste for soymilk among the masses .For this purpose the amount of flavoring agent should be increase in the starting and than decrease gradually after taste development but care should be taken never to disturb its natural white color.

REFERENCES

1. Ahmed, M., F. Muhammad and I.Khan.1983.Technological and nutritional evaluation of cereals, pulses and grain legumes. Annual Report Nuc. Inst. Food Agri. Peshawar: 55-57.
2. A.O.A.C. 1984. Official method of analysis. Assoc.Of Analytical Chemist.12th edition Washington D.C.
3. Al-Haq, M.I. 1988. Commercial manufacturer of pasteurized mango fruit flavored milk based drinks. B.Sc. (Hons).Deptt.of food Sci.and Tech. Univ. of. Agri. Faisalabad.
4. Altschul, A.M. 1965. Protein 1st Ed. Champon & Hall Ltd.London:219-22
5. Escuta, E.E. and J. Benson.1979. Comparative acceptability of soymilk produced by different processing methods. Philippine Agriculturist. 62:248-254.

6. Gorner,F., J. Jancekova and K.Nemcekova.1977. Changes in stored UHT milk. Prumys lPotravin 28(8):441-445.(DSA,40(5):2678;1978).
7. Gould, J.A.1946.Lactic acid in milk. IV. Effect of storage of evaporated milk on lactic and formic acid production on pH and on acidity and formula titration. J. Dairy Sci. 29(1):39.
8. Hussain S.A.H. 1986.The effect of mettalic contamination on the quality of raw milk. M.Sc.(Hons) Food Tech. Thesis. Univ. of Agri. Faisalabad.
9. Khaleque, A., W.R. Banatyne, and G.M.Wallace. 1970. Studies on the processing and properties of soymilk .J. Sci. Food and Agric.21;579-583.
10. Munir, S., M. Saeed and H.Bakhtiar.1985.Quality changes and acceptability of soymilk as affected by processing methods and storage. Sarhad. J. Agric. 1(1):129-132.
11. Nelson, I., M.P. Steenberg and L.S. Wei. 1978. Whole soybean food for home and village use. Int. J. Food Sci. 18(1): 452-465
12. Nsofor, L.M and E.U.Chukwu.1992.Sensory evaluation of soymilk –based yoghurt. J. Food Sci. Tech. Mysore.29 (5):301-303.
13. Rehman, A.1989.Development of milk based banana fruit beverage using cow's and buffalow"s milks. B.Sc. (Hons). Deptt. of Food Tech.University of Agri. Faisalabad.
14. Ward, J.L.1995. An American introduction to tofu. Accessed from: http://www.com/whatis.htmil.
15. Webb, B., H. Johnson and J.A. Alford.1974.Fundamentals of dairy chemistry. The AVI pub. CO. Inc. West port, connecticut, USA.
16. Wilkowski, H.H.1964.Relationship between titrable acidity and pH during Lactic acid fermentation in reconstituted non-fat-milk. J. Dairy Sci. 37(1):22-28

PAK. J. FOOD SCI., 22(3), 2012: 161-167
ISSN: 2226-5899

Characterization of lupin seed oils extracted from bitter and sweet types

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Abstract

Lupin seed oils extracted from bitter and sweet varieties were investigated for their physicochemical properties. There was no difference in the melting points of sweet and bitter types of oils. Iodine value of bitter lupin seed oil was higher than sweet type. There was a significant difference in the Saponification and ester value of both types of oils. The peroxide value of sweet oil was 1.97 ± 0.18 meq/Kg as compared to bitter type having 1.85 ± 0.21. The fatty acid profiles of the lupin seed oils showed that total unsaturated fatty acids were higher (11.35%) in bitter type than sweet i.e 9.63%. The predominant fatty acid in both oils was oleic acid. Its concentration in bitter oil was 52.22 ± 2.32% and in sweet oil was 44.93 ± 2.16%. Sweet lupin seed oil contained higher amount (42.06%) of total essential fatty acids as compared to bitter lupin seed oil (33.81%). Overall the amount of saturated fatty acids was lower than unsaturated fatty acids in both types of oils. Among tocopherols in both types, β fraction was not detected while γ fraction dominated. The other fraction i.e α tocopherol was significantly higher (5.41 ± 0.09 mg/100g oil) in bitter oil as compared to sweet type (4.96 ± 0.10 mg/100g oil). The oil classes like hydrocarbons (0.94 ± 0.06%), triglycerides (75.48 ± 0.87%), free fatty acids (9.26 ± 0.09%) and alcohols (0.55 ± 0.05) were higher in sweet lupin while steroids (4.32 ± 0.06%), diglycerides (7.79 ± 0.07%) and phospholipids (2.24 ± 0.08%) were higher in bitter lupin seed oil.

Key words: Lupin seed oil, bitter, sweet, fatty acids, oil classes

Introduction

Edible oils and fats are highly recognized for their dietary roles. They are the essential part of the human diet. Vegetable oils and fats have wide application in foods where they are used in frying, salad dressing, shortening of pasty, margarine, cooking and ice cream manufacture. World Health Organization (1994) recommended that 20-30% conversion rates from fat to energy are ideal to ensure good health. The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have recommended an average daily intake of 55 g fat per capita to compliment the requirement for energy (Kabyemela *et al.,* 1992). The lupin, a leguminous plant, has great potential as an oilseed crop in regions having a temperate climate (Hudson *et al.*, 1976). It is a valuable plant which is able to grow in different soils and climates. Lupin production is increasing, due to its potential as a source of protein, for pharmaceutical purposes and green manure (Gaultier *et al.,* 2003 and Farrell, 1999). The most commonly used part of the lupin is the seed. Lupin seeds are highly valuable as human food and animal feed, with high protein and dietary fibre (Petterson, 1998).White lupin, a sweet variety (*Lupinus albus L.)* is gaining growing interest worldwide for its high potential as human food and animal feed (Bhardwaj *et al*., 2004 ; Petterson, 2000). White lupin is one of the neglected legumes with the highest protein content and also the fat is like sesame with a great amount of oil (60%) (Ashri, 1994).These lupins are referred to as sweet lupins because they contain smaller amounts of toxic alkaloids than the bitter lupin varieties. The bitter lupin is an annual legume traditionally cultivated around the Mediterranean area (Huyghe, 1997). The bitter lupin seed is processed in a manner similar to other oilseeds (Cerletti and Duranti, 1979). Lupin seeds may also be a potential source of alimentary cellulose for the production of dietetic food. The high protein fraction (25-40%) could be used as a substance for enriching different kinds of products, such as pastries, breads, chips and milk substitutes and also be a main food component when animal proteins are eliminated (Erickson, 1988). The lupin oil can be used as human food because L*upinus albus* and L*upinus mutabilis* species contain an average oil content of 110 and 190 g/kg, respectively and are already identified as an oil sources (Oliveira and Ferreira, 2000; Gross *et al*., 1988). Williams (1989) reported that L*upinus mutabilis has* intermediate values of oleic and linoleic acids as compared to the olive and sunflower oils. Consumption of lupin based diets lowers plasma glucose, cholesterol, and triglycerides (Chango *et al.,* 1998). Lupin oils are characterized by a balanced fatty acid composition with total saturated FA of about 10% and total unsaturated FA around 90% (Petterson, 2000; Bhardwaj *et al*., 1998). The fat level in lupin is ranked third after groundnut and soybean among different legumes (Cowling *et al.,* 1998). Being one of the oil bearing plant materials, lupin seeds can be explored for the production of healthier dietary oil.

The main purpose of this paper is to characterize the oils extracted from sweet and bitter varieties of lupin seeds.

Materials and Methods

Materials

Lupin seeds of both varieties bitter (*Lupinus termis*) and sweet (*Lupinus albus*) were obtained from the local market of Riyadh, Saudi Arabia. The seeds were cleaned and render them free of dust, then stored in polyethylene bags in the refrigerator until used.

Preparation of lupin seed oils

Lupin seeds were crushed using household mill (Braun, Germany), and then defatted by soaking in n-hexane (*Boiling point* 67°C) for 48 hr with several changes of the solvent. Evaporation of hexane was performed using rotary evaporator (ROT. VAC. EVA. RVA. 64, Czechoslovakia) and the produced oil samples were stored separately in refrigerator inside dark tight stopper glass until analysis.

Physical properties of oils

The specific gravity of oil samples was determined using 10-ml pycnometer at 20°C according to the method of AOCS (1973). Refractive index of the oil samples was measured using a Refractometer (CARL ZEISS JENA, GDR) at 25°C according to the method of AOCS (1973). The melting point of the oil samples was estimated using a hot plate microscope having a thermometer (Model M M K. Germany). Temperature was adjusted to raise by 1°C every 2 min. The viscosity of oil samples was measured as Cm poise using viscometer ICI Co Research Equipment-London at 50°C. The color of oil samples was determined by a Lovibond Tintometer using a 5.25 inch cell. The yellow filter was fixed at 35 and the intensity of red was measured according to the AOCS (1973).

Chemical characterization of oils

The acid value (Cd 3a-63), peroxide value (Cd 8-53), saponification value (Cd 3-25), iodine value (Cd 1-25) and unsaponifiable matter (Ca 6a-40) of the oil samples were determined according to AOCS (1973).

Fatty acid composition

The methyl esters of crude oil were prepared according to Chalvardjian (1964), using 1% of H_2SO_4 in absolute methyl alcohol. A Perkin-Elmer gas chromatography (Model F22) with a flame ionization detector at 250°C was used in the presence of nitrogen as a carrier gas. A glass column (2 m x 2.5 mm) packed with Chrom Q 80/100 mesh at a temperature of 270°C was used. Standard fatty acids methyl esters were used for identification. The area under each peak was measured and the percentage expressed in regard to the total area.

Neutral lipid fractionation

This was carried out by thin layer chromatography using precoated plastic sheets (POLYGRAM SIL G, 0.25 mm silica gel, Made in Germany) according to the method of Mangold and Malins (1960). The plate developing solvent was petroleum ether, diethyl ether and acetic acid (70:30:2, v:v:v). The fractionated oil classes were visualized by exposing to iodine vapor in closed vessel and identified by comparing its R_f values with those reported in literature. The quantitative content of each separated fraction type on the plate was measured using the charring densitometry method (Blank *et al.,* 1964) and the area under each peak was measured by triangulation method.

Tocopherols

Saponification and extraction of tocopherols were done as described by Koning *et al.* (1996). Tocopherols were determined by HPLC using Hewlett-Packared apparatus model 1050, with UV detector, on Absorbosphere Silica column (4.6X250 mm, Alltech) with precolumn (Absorbosphere Silica, Alltech). Operation conditions for HPLC analysis were as follows: UV detection at 295 nm, ambient temperature, eluant-hexane/2-propanol (both Romil Chemicals) 98.5/1.5, flow rate 1 ml/min. α, β, γ and δ tocopherols standards (Merck, Germany) were used to identify peaks of chromatograms, and peak area was used to determine the tocopherols concentration by computer integration operated in the mode of external standard.

Statistical Analysis

Results are expressed as the mean value ± SD of three separate determinations. The data were statistically analyzed using analysis of variance (ANOVA) and least significant difference using SAS (1985). Significant differences between any two means were determined at the $P \leq 0.05$ level

Results and Discussion

Physicochemical properties of lupin seed oils

The results regarding the analyses of some of the physical attributes of bitter and sweet lupin seed oils are given in Table 1. All the physical attributes studied were not significantly different in both types of oils. The acid values (0.935 ± 0.07 and 0.853 ± 0.08) observed for bitter and sweet lupin seeds oil, respectively were lower as compared to others plant oils. There was no difference in the melting points of both types of oils i.e 5.2 ± 0.06 ºC. Rheological studies of oils indicated that there was a non significant difference between the viscosities of bitter and sweet types. Table 2 shows some of the chemical components of lupin seed oils. There was no significant difference in both types of oils with respect to peroxide value and unsaponifiable matter.

Table (1): Physical properties of bitter and sweet lupin seed oils*.

Properties	Lupin seed oils	
	Bitter	Sweet
Refractive index (25°C)	1.4683[a] ± 0.002	1.4685[a] ± 0.002
Specific gravity (20°C)	0.915[a] ±0.06	0.920[a] ± 0.07
Melting point (°C)	5.2[a] ± 0.06	5.2[a] ± 0.06
Viscosity (cP)	40.7[a] ± 0.15	40.8[a] ± 0.32
Color**	9.0 R/ 5.5 B	7.0 R/ 3.7 B

Means in the same row with different letters are significantly difference ($P < 0.05$).
* Means ± standard deviation of means of three determinations.
** Means of two determinations.

Table (2): Chemical properties of bitter and sweet lupin seed oils*.

Properties	Lupin seed oils	
	Bitter	Sweet
Acid value (mg KOH/ g oil)	0.935 [a] ± 0.07	0.853 [b]±0.08
Saponification value (mg/g)	192.92 [a] ± 1.70	187.90 [b] ± 1.35
Ester value (mg KOH/ g oil)	191.99 [a] ± 1.64	187.08 [b]±1.46
Peroxide value (meq/ kg)	1.85[a] ±0.21	1.97[a] ± 0.18
Iodine value (Hanus) (g I/ 100 g oil)	118.4 [a] ±2.06	108.7 [b] ± 2.15
Unsaponifiable matter (%)	3.51[a] ±0.24	3.66[a] ± 0.25

Means in the same row with different letters are significantly difference ($P < 0.05$).
* Means ± standard deviation of means of three determinations.

Table (3): Fatty acids composition of bitter and sweet lupin seed oils.

Fatty acids %	Lupin seed oils	
	Bitter	Sweet
Myristic ($C_{14:0}$)	0.10 [b] ± 0.02	0.2 1[a] ± 0.03
Palmitic ($C_{16:0}$)	9.41 [a] ± 0.18	7.71 [b] ± 0.19
Palmitoleic ($C_{16:1}$)	0.49 [b] ± 0.08	0.64 [a] ± 0.07
Stearic ($C_{18:0}$)	1.84 [a] ± 0.04	1.71 [b] ± 0.04
Oleic ($C_{18:1}$)	52.22 [a] ± 2.32	44.93 [b] ± 2.16
Linoleic ($C_{18:2}$)	20.51 [b] ± 0.84	26.25 [a] ± 0.75
Linolenic ($C_{18:3}$)	13.30 [b] ± 0.36	15.81 [a] ± 0.41
Arachidinic ($C_{20:1}$)	2.1 3 [b] ± 0.21	2.74 [a] ± 0.19
Total saturated fatty acid (TSFA)	11.35	9.63
Total unsaturated fatty acid (TUFA)	88.65	90.37
TUFA/ TSFA	7.78	9.38
Total essential fatty acid	33.81	42.06

Means in the same row with different letters are significantly difference ($P < 0.05$).
* Means ± standard deviation of means of three determinations.

Acid value (AV) is an important indicator of vegetable oil quality and is expressed as the amount of KOH (in milligrams) necessary to neutralize free fatty acids Saponification value and ester value were significantly lower in sweet lupin seed oil as compared to bitter lupin seed oil. The peroxide values of both oils recorded during the present study are well below the range of those reported by Codex Alimantarius Commission (2001) i.e <10 meq/ kg for soyabean, rapseed , cottonseed and coconut oils. The iodine values observed both in bitter (118.4 ± 2.06) and sweet (108.7 ± 2.15) lupin seed oils were higher than the earlier reported by Lauro (1934) who reported 96% Iodine value in two samples. The level of unsaponifiable matter in both types of oils was higher i.e 3.51 ± 0.24 and 3.66 ± 0.25% for bitter and sweet varieties, respectively. The earlier studies by Mohammed and Awatif (1998) showed that sesame oil of different types contains 1.1-1.3% unsaponifiable matter. It was also observed that higher levels of unsaponifiable matter increased the antioxidant activity of the oil (Konsoula *et al.,* 2010; Mohammed and Awatif, 1998 **).** It can be assumed that presence of higher levels of unsaponifiable matter in lupin seed oils can contribute to the more stability than other types of oils.

Fatty acid profile of lupin seed oils

Fatty acid composition of sweet and bitter lupin seed oils is presented in Table 3. There was a significant variation between two types of oils with respect to their fatty acid profiles. The dominant fractions in both oils were Oleic, Linoleic and Linolenic acid among other fatty acids. The oleic acid was significantly higher in bitter lupin oil (52.22 ± 2.32%) than sweet lupin seed oil (44.93 ± 2.16%). Sweet lupin seed oil had higher contents of Myrestic (0.2 1±0.03%), Palmitoleic (0.64 ± 0.07%), Linoelic (26.25 ±0.75%), Linolenic (15.81±0.41) and Arachidoic acids (15.81 ± 0.41%) as compared to bitter lupin seed oil in which these fractions were 0.10 ± 0.02%, 0.49 ± 0.08%, 20.51± 0.84%, 13.30 ± 0.36% and 2.1 3 ± 0.21%, respectively. The content of linolenic acid in both types of lupin seed oils are far below (less than 16%) than the contents reported in flaxseed oils i. e above 47% by (Zhang *et al.,* 2010). This can be a good indicator of the better stability of lupin seed oil as the presence of unsaturated fatty acids may cause problems during the long term storage of oil containing food products. The amounts of total unsaturated and essential fatty acids were higher than saturated fatty acids in both types of oils. The sweet lupin seed oil had higher contents (42.66%) of essential fatty acids as compared to bitter lupin seed oil. The results are comparable with an earlier study on lupin by (Bhardwaj *et al.*, 1998) who reported 51% Linoleic acid, 23% Oleic acid, 10% Palmitic acid, 7% Linolenic acid and 14% saturated fatty acids. The results further indicate that ratio of linoleic acid over linolenic was good in both sweet and bitter lupin seed oils. The medical research has shown that the excessive contained in 1 g of oil (Firestone, 1996; ISO 1996). The acid value was significantly higher in bitter lupin seed oil (0.935 ± 0.07) than sweet lupin seed oil (0.835 ± 0.08). level of linoleic acid relative to linolenic acid may increase the probabilities of a number of diseases (Hibbeln, 2006). However, it has been suggested by Hu (2001) that linoleic to linolenic acid ratio of 10 or less results in reduction of cardiovascular diseases. The saturated fatty acid contents of the bitter and sweet lupin seeds analyzed in the present study are lower (i.e 11.35 and 9.63%, respectively) as compared to earlier reported by Fitzpatrick and Scarth (1998) in soyabean oil (i.e. 14%). These findings can be helpful to recommend the use of lupin seed oil as a replacement of soyabean oil due to its higher contents of saturated fatty acids.

Oil classes of lupin seed oils

Data presented in Table 4 showed that the two types of lupin seeds oils differed significantly in their oil classes. The sweet lupin seed oil had significantly higher contents of hydrocarbons (0.94 ± 0.06), triglycerides (75.48 ± 0.87), free fatty acids (9.26 ± 0.09) and alcohols (0.55 ± 0.05) whereas bitter lupin seed oil had higher contents of steroids (4.32 ± 0.06), diglycerides (7.79 ± 0.07), monoglycerides (1.98 ± 0.06) and phospholipids (2.24 ± 0.08). Data further substantiated that triglycerides were prime class in both types of oils contributing above 73%. The results are comparable to the findings of Hamama and Bhardawaj (2004) who reported 2.6 to 2.8% phospholipids in seven varieties of white lupin seed oils. Phospholipids can also serve as an emulsifier in different food products. The presence of higher phospholipids content in lupin seed oil observed in present studies can also be an added benefit while using these oils.

Tocopherols

Tocopherols are the most popular plant antioxidants in the food industry. The natural antioxidants alpha-, beta-, gamma- and delta-, are compounds present in the green parts of plants and in their seeds (Sheppard *et al*., 1993). Each of these four types has different biological and antioxidant activities (Kamal-Eldin and Appelquist, 1996) but they act as the main antioxidants in cell membranes (Bramley *et al.,* 2000). Tocopherol contents of sweet and bitter lupin seed oils are shown in Table 5. Bitter lupin seed oil has significantly higher α- (5.41 ± 0.09) and *δ*-Tocopherol (4.23 ± 0.14) contents than sweet lupin seed oil. The *γ*-Tocopherol fraction was not detected while *β*-Tocopherol was not significantly different in both types of oils. The total tocopherol contents were higher (79.51%) in bitter lupin seed oil when compared to the oil of sweet lupin seed (63.58%). The results suggest that lupin vitamin E content is similar to that of soybean. Lampart-Szczapaa *et al.* (2003) also reported the three tocopherols in *L. albus* as alpha- (2.8%), Beta- (86.1%), Delta (11.1%) and in *L. luteus* as alpha- (3.2%), Beta- (88.5%) and Delta (8.3%).

Table (4): Oil classes of bitter and sweet lupin seed oils*.

Oil classes (%)	Lupin seed oils	
	Bitter	Sweet
Hydrocarbons	0.69 [b] ± 0.07	0.94 [a] ±0.06
Triglycerides	73.96 [b] ± 0.96	75.48 [a] ±0.87
Free fatty acids	8.71 [b] ±0.08	9.26 [a] ±0.09
Steroids	4.32 [a] ± 0.06	4.11 [b] ±0.05
Diglycerides	7.79 [a] ± 0.07	7.23 [b] ±0.08
Monoglycerides	1.98 [a] ±0.06	1.25 [b] ±0.06
Alcohols	0.31 [b] ±0.03	0.55 [a] ± 0.05
Phospholipids	2.24 [a] ±0.08	1.18± 0.07

Means in the same row with different letters are significantly difference ($P < 0.05$).
* Means ± standard deviation of means of three determinations.

Table (5): Tocopherol fractions content of bitter and sweet lupin seed oils (mg/ 100 gm oil)*.

Fractions	Lupin seed oils	
	Bitter	Sweet
α-Tocopherol	5.41[a] ±0.09	4.96[b] ±0.10
β-Tocopherol	ND	ND
γ-Tocopherol	69.93 [a] ±1.16	54.52 [b] ±0.94
δ-Tocopherol	4.23[a] ± 0.14	4.10[a] ±0.13
Total tocopherol	79.57	63.58

Means in the same row with different letters are significantly difference ($P < 0.05$).
* Means ± standard deviation of means of three determinations.
ND = Not detected
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But according to Hatzold *et al.* (1983) only two fractions i.e alpha- (3.8%) and gamma-tocopherol (96.2%) were present in lupin seed oil.

Conclusion

Based on the present studies conducted to characterize the lupin seed oil of bitter and sweet varieties, it can be concluded that this oil can be used as a healthy replacement of other traditional oils used for dietary purposes. The balanced fatty acid profile and presence of antioxidants makes these oils suitable for their utilization in different food products.

References

1. AOCS. 1973. Official and Tentative Methods of the American Oil Chemist's Society. Vo. 1 (3rd ed.) AOCS, Champaign, IL.
2. Ashri, A. 1994 Genetic resources of sesame: present and future perspectives In: biodiversity in Asia: conservation, evaluation and improvement Sesame. R.K. Arora., K.W. Riley (Eds), pp. 25–39. New Delhi , India.
3. Bhardwaj, H.L., Hamama, A. A. and Merrick L.C. 1998. Genotypic and Environmental Effects on Lupin Seed Composition, Plant Foods for Human Nutrition, 53:1-13.
4. Bhardwaj, H.L., Hamama, A. A.., van Santen, E. 2004. White Lupin Performance and Nutritional Value as Affected by Planting Date and Row Spacing. Agronomy Journal, 96:580–583.
5. Blank, M. L.; Schmidt, J. A., Privett, O. S. 1964. Quantitative analysis of lipid by thin-layer chromatography. Journal of American Oil Chemists Society, 41: 371- 375.
6. Bramley, P. M., Elmadfa, I., Kafatos, A., Kelly, F. J., Manios, Y., Rexborough, H. E., Schuch, W., Sheehy, P. J. A., Wagner, K.H. 2000. Vitamin E-review. Journal of Science and Food Agriculture, 80, 913–938.
7. Codex Alimentarius Commision. 2001. Codex Alimentarius-Joint FAO/WHO Food Standards Programme, Fats, Oils and Related Products, 2nd edn. (rev. Vol. 8 (available at http://www.fao.org/docrep/004/y2774e/y2774e04.htm#bm4.1 / (accessed Jan 29, 2011).
8. Cerletti, P., Duronti M. 1979. Development of the Lupin Proteins. Journal of American Oil Chemist Society, 56:460-466.
9. Chalvardjian, A. M. 1964. Fatty acids of brown and yellow fat in rats. Biochemistry Journal, 90: 518-521.
10. Chango, A., Villaume C., Bau H.M., Schwertz A., Nicolas J., Mejean, L. 1998. Effects of Casein, Sweet White Lupin and Sweet Yellow Lupin Diet on Cholesterol Metabolism in Rats. Journal of Science of Food and Agriculture, 76:301-309.
11. Cowling, W. A., Buirchell, B. J., Tapia, M. E. 1998. Lupin. *Lupinus L*. Promoting the conservation and use of underutilized and neglected crops (Vol. 23). Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Research Institute. Rome, Italy, 105pp.
12. Erickson, D.R .1988. Chemical characteristics of soybean oils. In: Soybean Utilization Alternatives. The Center for Alternative Crops and Products. L. McCann (ed),. University of Minnesota, St. Paul, MN. USA.
13. FAO/WHO. 1994. Fats and oils in human nutrition Report of Expert Committee, Food and Nutrition Paper No.57, FAO, Rome, Italy.
14. Farrell, D. J., Perez-Maldonado, R. A., Mannion, P. F. 1999. Optimum inclusion of field peas, faba beans, chick peas and sweet lupins in poultry diets. II. Broiler experiments. British Poultry Science, 40(5), 674–680.
15. Firestone D. (Ed.). 1996. Method Ca 5a–40. Free Fatty Acids. Official Methods and Recommended Practices of the American Oil Chemists Society, 4th ed., American Oil Chemists Society, Champaign, USA.
16. Fitzpatrick, K. and. Scarth, R.1998. Improving the health and nutritional value of seed oils. PBI Bulletin. NRC-CRC. January: 15-19.
17. Gaultier, F., Foucault-Bertaud, A., Lamy, E., Ejeil, A. L., Dridi, S. M., Piccardi, N., *et al*. 2003. Effects of a vegetable extract from *Lupinus albus* (LU105) on the production of matrix metalloproteinase (MMP1, MMP2, MMP9) and tissue inhibitor of metalloproteinases (TIMP1, TIMP2) by human gingival fibroblasts in culture. Clinical Oral Investigation, 7(4), 198–205.
18. Gross R., Von Baer, E., Koch, F., Marquard, R., Trugo, I., Wink, M. 1988. Chemical composition of a new variety of the andean lupin (*Lupinus mutabilis cv. Inti*) with low-alkaloid content. Journal of Food Composition and Analysis, 1: 1353–1361.
19. Hamama, A.A., Bhardwaj, H. L. 2004. Phytosterols, Triterpene Alcohols, and Phospholipids in Seed Oil from White Lupin. . Journal of the American Oil Chemists' Society, 81(11). 1039-1044.
20. Hatzold, T., Elmadfa, I., Gross, R. 1983. Edible oil and protein concentrate from *Lupinus*

mutabilis. Plant. Foods for Human Nutrition, 32, 125–132.

21. Hibbeln, J.R. 2006. Healthy intakes of n−3 and n−6 fatty acids: estimations considering worldwide diversity. American Journal of Clinical Nutrition, 83(6):1483-1493
22. Hu, F.B. 2001. The balance between omega-6 and omega-3 fatty acids and the risk of coronary heart disease. Nutrition, 17(9):741-742.
23. Hudson, B.J.F., Fleetwood, J.G., Zand-Moghaddam, A. 1976. Lupin: an arable food crop of temperate climates. Plant Foods for Man, 2:81-90.
24. Huyghe, C. 1997. White lupin (Lupinus albus L.). Field Crops Research, 3, 147–160.
25. ISO. 660. 1996. Animal and vegetable fats and oils - Determination of acid value and acidity International Organization for Standardization / 01-May-1996 / 8 pages.
26. Kabyemela, J.K., Mugyabuso, J., Kimenya, F,L. 1992. The National Vegetable Oil and Protein System. A Status Report Prepared for 1st National Oil Crop Workshop by Tanzania Food and nutrition Centre (TFNC), Tanzania.
27. Kamal-Eldin, A.., Appelquist, L.A. 1996. The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids, 31(7), 671 - 701.
28. Koning, E. J. M., Roomans, H. H. S., Beljaars, P. R. 1996. Liquid chromatographic determination of tocopherols and tocotrinols in margarine, infant foods and vegetable. Journal of the American Oil Chemists' Society, 79: 902- 906.
29. Konsoula, Z., Liakopoulou-Kyriakides, M. 2010. Effect of endogenous antioxidants of sesame seeds and sesame oil to the thermal stability of edible vegetable oils. LWT - Food Science and Technology, 43. 1379-1386.
30. Lampart-Szczapaa, E., Korczakb, J., Nogala-Kaluckaa, M., Zawirska-Wojtasiakc, R. 2003. Antioxidant properties of lupin seed products. Food Chemistry, 83, 279–285.
31. Lauro, M. F. 1934. The white lupine flour and oil. Journal of the American Oil Chemists' Society, 11(9): 196-197.
32. Mangold, H. K.., MaIms, D. C. 1960. Fractionation of fats, oils, and waxes on thin layers of silicic acid. Journal of American Oil Chemist Society, 37:383-385.
33. Mohamed, H. M. A., Awatif, I. I. 1998. The use of sesame oil unsaponifiable matter as a natural antioxidant. Food Chemistry, 62 (3): 269-276.
34. Oliveira, M. B. P. P., Ferreira, M. A. 2000. Study of Lupinus *(L. albus)* seed oil. Rev Port Farm, 38. 25–39.
35. Petterson, D. S. 1998. Composition and food uses of lupins. In: Lupins as Crop Plants. Biology, Production and utilization ,J. S. Gladstone, J. S. Atkins, and C. Hamblin (Eds.), pp. 356-358. Cab International, New York.
36. Petterson, D.S. 2000. The Use of Lupins in Feeding Systems-Review. Asian Australian Journal of Animal Sciences, 13:861-882.
37. SAS. 1985. SAS Users Guide: Statistics, version 5 ed.; SAS Institute: Cary, NC, USA.
38. Sheppard, L.J., Cape, J.N., Leith, I.D. 1993. Influence of acidic mist on frost hardiness and nutrient concentrations in red spruce seedlings. New Phytologist, 124. 595-605.
39. WHO. 1994. Fats and oils in human nutrition Report of a Joint FAO/WHO Expert Consultation Committee, Rome, Italy, 19-26 October 1993, World Health Organization, Geneva.
40. Williams W. 1989. Lupins. Biologist. 36, 177-181.
41. Zhang Z. S., Wang L. J., Li D., Li S. J., Zkan N. 2010. Characteristics of flaxseed oil from two different flax plants. International Journal of Food Properties, Volume 99999, Issue 1.

PAK. J. FOOD SCI., 22(3), 2012: 167-169
ISSN: 2226-5899

Incidence of *Staphylococcus aureus* contamination of marketed Processed Chicken products with special reference to its antibiotics sensitivity collected from Al Baha city markets, Saudi Arabia

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ABSTRACT

Staphylococci were isolated from marketed frozen processed chicken products in Al Baha city, Saudi Arabia. Twelve strains of the *Staphylococcus aureus* isolates were found to be enterotoxigenic. The enterotoxin types were Staphylococcal enterotoxin A (SEA) (10 strains) and Staphylococcal enterotoxin C (SEC) (2 strains). The isolates were found to be resistant to commonly used antibiotics. These findings pose health hazard to people eating these frozen chicken products and hence exposed to the risk of gastrointestinal disorders.

Keywords: Staphylococci, chicken processed products, antibiotic resistance, enterotoxin

INTRODUCTION

Staphylococcus aureus is an opportunistic pathogen which has emerged as a significant cause of nosocomial infections. In the recent years the incidence of community-acquired infections associated with this organism has been on the increase (Chambers, 2001). Food-borne illnesses in human beings due to bacterial pathogens and their toxins are well known all over the world (Hazariwala *et al*., 2002). Food-borne illness leads to a substantial economic and quality of life by a way of acute morbidity and chronic sequels (Duff *et al*., 2003). Staphylococcal intoxication is a leading cause of food borne intoxication and enterotoxigenic. Staphylococcus strains have been isolated from foods implicated in illnesses (Adesiyun 1995; Cencil *et al*.2003)

Little information is available on the antibiotic resistance survey and enterotoxin production potential of *Staph. aureus* isolated from poultry food products. It is very essential therefore to know the distribution of antibiotic resistant and enterotoxigenic strains of *Staph aureus* in any environment because of its public health importance. The antibiotic resistance patterns of isolates from clinical samples have been studied extensively (Ako-nai, 2005). Food borne illnesses in human beings due to bacterial pathogens and their toxins (Hazariwala *et al*., 2002).

Microorganisms especially yeasts are generally not considered important in the spoilage of meat products since their numbers in these products are highly variable relative to bacterial numbers (Jay and Margitic, 1981).

This study has been undertaken to evaluate the prevalence of antibiotic resistant and enterotoxin producing staphylococci from some frozen poultry products from Al Baha, Saudi Arabia.

MATERIALS AND METHODS

1. **Collection of samples**

A total of 20 random samples of frozen chicken products, represented by chicken fillets, chicken burgers and chicken luncheons (20 of each) were collected from different markets and supermarkets at Albaha city, Saudi Arabia. The samples were transferred to the laboratory in an ice box without undue delay.

Total bacterial count:

One gram of each sample was weighed thoroughly homogenized under aseptic conditions and 9 ml of sterile distilled water. This was transferred to a test-tube followed by serial dilution to 10^{-7} dilution. To determine total viable counts, 0.5 ml of each 10^{-5} and 10^{-7} dilutions were plated on Trypton Soy agar (Oxoid, UK) plates in triplicates for determination of the total bacterial counts. The plates were incubated at 37 ^{o}C for 24 hours.

***Staphylococcus aureus* count:**

The total bacterial counts were determined by using duplicate plates containing Blood Agar (BA) and Mannitol Salt Agar (MSA), which was inoculated with 0.1 ml of food homogenate sample from each dilution and were plated on and incubated at 37^{o}C. To confirm the identity of the isolates of staphylococci, isolates were subjected to morphological and biochemical studies using tests such as Gram staining, catalase, coagulase, carbohydrate fermentation, DNase and phosphatase tests as outlined in Barrow and Feltham (Barrow and Feltham, 1993) and Bergey's Manual (Holt *et al*., 1994). Other isolates recovered were also characterised following standard procedures outlined in the above manuals.

Antibiotic sensitivity testing

Antimicrobial sensitivity testing was performed on the identified staphylococci isolates by the Kirby-Bauer's disc diffusion method (Bauer *et al*., 1966). The antibiotics tested include: penicillin, gentamicin, oxacillin, ciprofloxacin, cotrimoxazole, clindamycin, erythromycin, vancomycin, amoxycillin, rifampicin, chloramphenicol, Ticarcillin and streptomycin. Oxacillin sensitivity was performed on Mueller-Hinton

agar supplemented with 4% NaCl, following standard method (National Committee for laboratory standards 2004). *Staph. aureus* strains are reported to be susceptible to oxacillin when its minimum inhibitory concentration (MIC) is ≤ 2mg/ml and resistant when its MIC is ≥ 4mg/ml. In addition, a 5µg methicillin disc (Oxoid, U.K) was used to confirm the resistance or otherwise to methicillin. Strains of Staph. aureus that were resistant to oxacillin were tested for beta-lactamase production and for their ability to produce enterotoxins (Efuntoye and Amuzat, 2007)

RESULTS AND DISCUSSION

The mean concentrations of total bacteria as recorded on the studied chicken products were 2.80 x 10^6, 4 x 10^8 and 3.72 x 10^7per gm of the collected luncheon, burger and fillet samples, respectively (Table 1). The mean concentration of *Staph aureus* were between 1.2 x 10^7 cfu/gm with the highest value recorded for chiecken burger and the lowest in chicken fillet (1.6 x 10^5 cfu/gm) (Table 2). The results revealed that staphylococci were isolated from all collected samples of chicken products. The presence of *Staph.aureus* may be as a result from handling or processing of these products and/ or during storage, where it is a common organism on the skin, hands and boil (Kuku 1985). The presence of Staphylococci in high numbers in cured meat may indicate the presence of enterotoxin-reproducing strains of *Staph. aureus* (AS/NZS, 1999), so it is important to keep on eye, evaluate, and detect food borne diseases outbreaks early to ensure the food safety. Moreover, the risk of exposure to staphylococci via the these chicken products was heightened by the facts that some of the isolated strains were coagulase positive, enterotoxin producers and hence potential disease-causing strains. The major enterotoxin encounter in this study is staphylococcal enterotoxin A (SEA), which is associated with staphylococcal food poisoning.

The resistant rates of the staphylococci from the chicken products food samples to different antibiotics are shown in Table (3). The isolates of *Staph. aureus* were susceptible to a large extent to rifampicin, vancomycin, amoxycillin, cotrimoxazole, ciprofloxacin, chloramphenicol and gentamicin. Majority were resistant to penicillin, oxacillin and clindamycin and 96.9% produce beta-lactamase enzyme.

The emergence of community-acquired *Staph. aureus* strains which are virulent and which possess resistance to antibiotics, particularly methicillin has been reported (Baba *et al*.2002). The net conclusion of this is that there is urgent need for continued microbiological evaluation of frozen chicken products to assess their sanitary quality to avoid outbreak of these disease. Whereas bacterial organisms could rapidly develop antibiotics resistant.

Table 1. Bacterial count from chicken products Samples collected from Supermarket in Al Baha city (n=20)

Sampling location designate	No of samples	Mean count (cfu/ml)*
Fillet	20	3.72 X 10^7
Burger	20	4.00 X 10^8
Luncheon	20	2.80 X 10^6

*Reading is the mean ± Standard error

Table 2. Average concentration (cfu/g.) of *Staphylococcus aureus* from chicken products Samples collected from Supermarket in Al Baha city

Sampling location designate	Time of collection (No of samples)	Mean count of staphylococci*
Fillet	20	1.6 x 10^5
Burger	20	1.2 x 10^7
Luncheon	20	1.47 x 10^6

*Reading is the mean ± Standard error

Table 3. Rates of resistance of *Staphylococcus aureus* isolated from chicken products Samples collected from Supermarket in Al Baha city

Antibiotic	*Staphylococcus aureus* (n = 30) No. (%)
Rifampicin(10µg)	1 (3.3)
Ticarcillin (5µg)	18 (60.0)
Ciprofloxacin(5µg)	7 (23.3)
Penicillin(10i.u)	29 (96.6)
Oxacillin(5µg)	23 (76.7)
Gentamicin(10µg)	8 (26.7)
Clindamycin(5µg)	21 (70.0)
Erythromycin(5µg)	9 (30.0)
Vancomycin(30µg)	2 (6.7)
Amoxycillin(10µg)	5 (16.7)
Streptomycin(10µg)	10 (33.3)
Chloramphenicol(30µg)	6 (20.0)
Cotrimoxazole(25µg)	6 (20.0)

REFERENCES

1. Ako-nai, A.K., F.M. Adeyemi, A.O. Aboderin, O.O. Kassim. 2005. Antibiotic resistance profile of staphylococci from clinical sources recovered from infants. African J. Biotech. **4**(8): 816-822.
2. Australian/New Zealand standards methods for food microbiology. 1999. Guide to determine the equivalence of food microbiology nest method. AS/NZS 4659.
3. Baba, T. 2002: Genome and virulent determinant of high virulence community-acquired MRSA. *Lancet.* 359: 1819-1827
4. Barrow, G.I. and R.K.A. Feltham. 1993: Cowan and Steel's Manual for Identification of Medical bacteria. Cambridge University Press, Cambridge
5. Bauer, A.W., Q.M.M. Kirby, J.K. Sherris and M. Turck. 1966. Antibiotic Bergey's manual of determinative bacteriology, 19th edition, William & Wilkins, Baltimore, USA
6. Cencil, G.B.T., M. Karama, P.V. Rossitto, R.A. Morgante and J.S. Cullor. 2003. Enterotoxin production by Staphylococcus aureus isolated from mastitic cows. J. Food Protection. **66** (9):1693-1696.
7. Chambers, H F. 2001: The changing epidemiology of *Staphylococcus aureus?* Emerg. Infect. Dis. **7**(2): 178-182.
8. Duff, S.B., E.A. Scott, M.S. Mafilios, E.C. Todd, L.R. Krilo, A.M. Gedded and S.J. Ackerman. 2003. Cost effectiveness of a targeted disinfection program in household kitchens to prevent food-borne illnesses in the United States, Canada and the United Kingdom. Journal of food protection bb (II): 2103-2115.
9. Efuntoye, M.O. and M.A. Amuzat. 2007. Beta-lactamase production by from infants. African J. Biotech. 4(8): 816-822.
10. Hazariwala, A., Q. Sanders, C.R. Hudson, C. Hofacre, S.G. Thayer and J.J. Maurer. 2002. Distribution of Staphylococci enterotoxin genes among *Staphylococcus aureus* isolated from poultry and humans with invasive staphylococcal disease. Avian Diseases. 46 (1): 132-136.
11. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Stanley and S.T. Williams. 1994. Identification of Medical bacteria. Cambridge University Press, Cambridge.
12. Jay, M.J. and S. Margitic. 1981: Incidence of yeasts in fresh ground beef and their ratios to bacteria. J. Food Sci. 46:648-649
13. Kuku , F.O. 1985. Spoilage of fruits, vegetable and tuber crop. Nigeria Food J. 2: 1-3.
14. National Committee for Clinical Laboratory Standards. 2004. Performance standards for antimicrobial susceptibility testing. NCCLS approved standard M100- S14, Wayne, PA. USA

PAK. J. FOOD SCI., 22(3), 2012: 171-179
ISSN: 2226-5899

Yeast, its types and role in fermentation during bread making process-A Review

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Abstract

The art of bread making goes back to very early stages of different historical eras. Bread is an important part of the human diet, but for many people, it is much more than just providing nutrients. Bread making can be a creative art—especially for the persons dealing with yeast breads. Many people enjoy creating beautiful and unique breads from yeast dough. The purpose of any leavener is to produce the gas that makes bread to rise during fermentation. Yeast does this by feeding on sugars in flour, and expelling carbon dioxide in the process. As the yeast feeds on sugar, it produces carbon dioxide. With no place to go but up, this gas slowly fills the balloon. A very similar process happens as bread rises. Carbon dioxide from yeast fills thousands of balloon-like bubbles in the dough. Once the bread has baked, this is what gives the loaf its airy texture. The present study will mainly deal with different types of yeast, its properties and different mathematical models to describe the dough behavior during fermentation.

Keywords: Yeasts, fermentation, dough behavior, mathematical modeling

Introduction

Yeasts are actually microbial eukaryotes which belong to ascomycetes that are good source of vitamin B and protein. Yeasts are plant-like unicellular fungi thriving on every living organism. Being living organism fungi require warmth, water, albumen or nitrogenous material and sugars to remain alive (The Artisan, The Yeast Treatise, 2002).

Yeasts are typically spherical, oval or cylindrical in shape and a single cell of *Sacharomyces. Cerevisiae* (a mold which ferments the sugar in cereal) is around 8 µm in diameter. Every cell has a double-layered wall, which is porous to certain substances and in this way food fabric is taken into the cell and metabolites leave it. Yeast is made up of many tiny, single-celled plants, which grow by budding and each bud breaking away from the parent cell and forming new buds. Though most yeast replicate only as single cells, under a number of circumstances some yeasts can figure out as filaments.

The conditions required for growth are warmth (optimum 25-30 °C), moisture and food (starch plus a small amount of sugar). Refrigeration slows down the growth so that yeast can be kept for a limited period of time. When the yeast is used, the conditions and the utensils should be kept lukewarm to obtain the best results. As soon as the yeast has been added to the dough or batter, the yeast begins to feed on the starch in the mixture, forming sugar, alcohol and carbon dioxide. The bubbles of CO_2 cause the dough to expand. The dough must be "kneaded" thoroughly to distribute the bubbles evenly and then left to rise again, usually to about double its original volume. If the mixture is left too long, acid produced by the oxidation of the alcohol results in taste sour of the product.

Yeasts thrive in habitats where sugars are present, such as fruits, flowers and bark of trees. However, saleable yeasts of today are fairly different from wild strains due to genetic treatment, allowing them to grow in inappropriate situations. The enzymes which are created by the yeast cells and act as natal catalysts in the fermentation process are maltase, invertase and zymase complex. Maltase has the aptitude to alter maltose, which is formed by starch degradation by alpha- and beta-amylases, to glucose and acts when the supply of simple sugars has been bushed. Invertase converts sucrose to glucose and fructose, while the doings of the zymase complex fallouts in the change of glucose, fructose and other simple sugars into carbon dioxide and ethanol. It is the carbon dioxide which raises the dough during fermentation (Madigan *et al.,* 2003).

Types of yeast

Baker's yeast is a commercial preparation consisting of dried cells of one or more strains of the fungus *Saccharomyces cerevisiae*. Bakers use yeast as a leavening agent in the rising of dough for baking. A secondary contribution of yeast to bread is flavouring and aroma. Bakers yeast is a high volume, low value product, with 1574 x 106 kg being produced per annum on a global scale (O'Shea, 2005)

Baker's yeast is marketed in two ways, either as compressed cakes or as a dry powder, however there is also a saleable intermediate of the process known as 'cream yeast'. Process considerations include media formulation (which has to be cost effective), and the limited respiratory capacity of yeast, which inhibits the production of biomass in favour of ethanol production. The fermentation of bakers yeast is strongly directed towards maximum biomass production, no byproducts

such as ethanol are desired and so the fermentations are sectioned to obtain this maximum biomass (Van Hoek *et al.,* 2003).

Now a days, baker's yeast is a product of biochemical, microbiological, technical knowledge and experience. Biochemistry has led to an insight into the fermentation process; microbiology has made it possible to breed new and better strains of yeast and to develop better techniques for sterilization and disinfection. Advanced technologies have led to the large scale production of yeast with a high degree of automation and process control, giving commercial yeast of consistent quality and activity at an economic price. By feeding on sugars from the starch in flour, yeast produces carbon dioxide. This gas expands the gluten proteins in the flour and causes the dough to rise, this process of bread making being the most commonly associated with yeast. Scientists now cultivate strains of bakers yeast for their ability to make dough rise and produce loaves of good height, texture and flavour.

Cream yeast is not typically termed a 'bakers yeast product' but is relevant as it represents a major step in the process and is a marketable product itself. At the end of the fermentation, the fermentor/yeast broth is concentrated using a series of combined centrifugation and washing steps, into a yeast cream with a solids concentration of approximately 20 %. The yeast is then cooled to approximately 4 °C, an ideal temperature to restrict the growth of any contaminating mesophilic microorganisms. The cooled yeast cream is stored in a stainless steel cream tank, which is insulated and equipped with agitators and cooling pipes (Kristiansen, 1994). Effectively preventing heat exchange with the surrounding atmosphere, keeping the cream at 4 °C. Following storage either of two pathways can be followed. The first involves the preparation for sale of the cream yeast itself. Cream yeast is basically the liquid product and can therefore be transferred into sterile tanks/containers and distributed to bakeries, where it is used to produce yeast based products. The advantage of cream yeast is that it excludes any human handling thus reducing the risk of contamination by handling, however due to its high (water) volume, transport costs can be expensive. For this reason, distribution is generally confined to a particular area (Lallemand, 2001).

Granular yeast, also known as instant dried yeast, is a form of compressed yeast. Stored cream/liquid yeast is passed through a filter, usually a filter press or rotary vacuum filter, which removes water increasing its solids content to approximately 30 %. Salt may also be added to the cream yeast prior to filtration to aid the removal of water. The filtered yeast is then dried using fluid-bed dryers. As the yeast is dry it generally does not require refrigeration as the low water content reduces the risk of microbial contamination. Emulsifiers and oils can be added at this point to texturize the yeast and aid the cutting process. As the name implies, granular yeast is crumbled into granules, the granulation process being carried out by a granulator. Granular broths are typically used to make restoring drinks to serve in a cup; the practicality of granular products coming both from their instantly soluble nature and the fact that they are easily measured (Bauer, 2005).

The filtered and dried yeast can alternatively be used to make cake yeast. Cake yeast is another form of compressed yeast and can be categorized as active dry yeast. It differs from granular yeast in that rather than granulation, the dried yeast is extruded or cut into blocks/cakes. Similar to granular yeast cake yeast also contains about 30 % solids (70 % water). The composition of solids may vary depending on the growth rate of the yeast as lower growth rates give lower protein, lower activity, higher carbohydrate, and higher stability (Lallemand, 2001)

Properties of yeast

Preservatives are commonly used in breads because economic losses from bread spoilage caused by bacteria or by moulds are substantial. Ropy spoilage is caused mainly by *Bacillus subtilis* and *Bacillus licheniformis*, the spores of which contaminate raw materials such as flour, bread improvers, yeast, etc., and survive baking temperatures (Rosenkvist and Hansen, 1995).

Ropy spoilage in bread is first detected by an odour similar to that of pineapple. Later, the crumb becomes discoloured, soft and sticky to the touch, which makes the bread inedible. The deterioration of bread texture is due to slime being formed as a result of the combined effect of the proteolytic and amylolytic enzymes produced by some bacillus strains that results in slime formation (Viljoen and von Holy, 1997; Sorokulova *et al.,* 2003). The full extent of losses caused by ropy spoilage of bread is difficult to quantify, because the condition is often misidentified as sour or rotten spoilage caused by failed dough leavening or an insufficient bake. Consumption of ropy bread may cause illness if bacteria are present at ≥108 cfu/g (Kramer and Gilbert, 1989; Rosenkvist and Hansen, 1995). Ropiness can develop very rapidly under warm and humid conditions, so it is a common problem in the warm climates of Mediterranean countries, Africa and Australia (Voysey and Hammond, 1993). Bacillus spore numbers can be controlled by ensuring raw material quality, good sanitation and cooling of production and storage environments (Viljoen and von Holy, 1997). Spore germination and growth in bread can be inhibited by chemical preservatives such as propionic and acetic acids, although the current trend is to reduce the levels of these substances (Pattison *et al.,* 2004; Marin *et al.,* 2002). Acetic acid adversely affects the organoleptic quality of baked products, while propionic acid has been reported to cause irritability, restlessness, inattention and sleep disturbance in some children (Dengate and Ruben,

2002; Spicher, 1983). Alternative antimicrobial systems to prevent bread spoilage are therefore required.

The yeast used for bread manufacturing is *Saccharomyces cerevisiae*, often referred to as simply baker's yeast. It converts the fermentable sugars present in the dough into carbon dioxide and ethanol as the main products. The fermentation intensity depends on the form of the yeast and the availability of fermentable sugars in the flour, including maltose produced by starch hydrolysis (Hutkins, 2006).

During the bread-making process, baker's yeast (mostly strains of *Saccharomyces cerevisiae*) is uncovered to many environmental stresses such as air-drying, freeze–thaw, and high-sucrose concentrations (Attfield, 1997). Yeast cells worn for bread making must acclimatize to different sucrose concentrations during dough-fermentation processes (Tanaka *et al.,* 2006). In exacting, sweet dough (high-sugar dough) contains up to roughly 30 % sucrose per weight of flour. Such high-sucrose concentrations apply harsh osmotic stress that badly damages cellular mechanism (Verstrepen *et al.,* 2004) and hold back the optimal fermentation aptitude of yeast. To evade lethal injury, baker's yeast cells want to get osmotolerance, but the progress of osmotolerant baker's yeast strains will require knowledge of the molecular mechanism concerned in high-sucrose stress lenience, for example, by the introduction of stress proteins, the buildup of stress protectants, and the variations in membrane composition (Shima & Takagi, 2009).

When elevated osmotic pressure is felt, *S. cerevisiae* cells collect glycerol and trehalose (Cronwright *et al.,* 2002; De Virgilio *et al.,* 1994; Hino *et al.,* 1990; Hirasawa *et al.,* 2006; Shima *et al.,* 1999). Microarray examination and genome-wide screening using a removal strain group exposed that the metabolism of glycerol and trehalose, both of which are recognized as osmoprotectants, is significant for high-sucrose stress tolerance (Ando *et al.,* 2006; Tanaka-Tsuno *et al.,* 2007). In reply to osmotic stress, proline is accumulated in many plant and bacterial
cells as an osmoprotectant (Csonka, 1981; Verbruggen & Hermans, 2008). During a variety of stresses, yeast cells encourage glycerol or trehalose production, but the intracellular proline level is not augmented under a range of stress circumstances (Kaino & Takagi, 2008). Proline has many functions in vitro, such as protein and membrane stabilization, decreasing the Tm of DNA, and scavenging of hasty oxygen species (ROS), but the mechanisms of these functions in vivo are not well understood (Takagi, 2008). *Sacharomyces cerevisiae* cells that collect proline, and the engineered strains effectively indicated improved lenience to many stresses, counting freezing, desiccation, oxidation and ethanol (Matsuura & Takagi, 2005; Morita *et al.,* 2002; Takagi *et al.,* 1997; Takagi *et al.,* 2000; Takagi *et al.,* 2005; Terao *et al.,* 2003). With respect to high osmotic pressure, it was found that the proline oxidase-deficient strain, which had a considerably elevated proline level, was obviously more osmotolerant than were other strains in the existence of 1 M NaCl (Takagi *et al.,* 1997). Recently, it was found that proline accumulating baker's yeast retained higher-level fermentation aptitude in the frozen dough than that of the wild-type strain (Kaino *et al.,* 2008). Based on these results, it is concluded that it is possible that proline collection confers tolerance to high-sucrose stress on baker's yeast. For the application of recombinant yeasts for marketable use, self-cloning yeast that has no foreign genes or DNA sequences apart from yeast DNA might be more satisfactory for consumers than a genetically modified yeast.

There is no doubt that folate (vitamin B9) has a vital role in primary cell processes, such as nucleic acid and amino acid biosynthesis. Inadequate folate intake may lead to the typical folate insufficiency disease megaloblastic anaemia (Wickramasinghe, 2006) and greater risks for neural tube defects (Berry *et al.,* 1999; Wald *et al.,* 1996) as well as other malformations (Lucock, 2000). In addition, the useful role of folate for more than a few other diseases such as cardiovascular diseases (Brouwer *et al.,* 1999), Alzheimer's disease (Seshadri *et al.,* 2002) and some forms of cancer (Choi and Mason, 2000) is under careful examination.

Humans are, in contrast to yeasts and plants, auxotrophic for this vitamin and must therefore satisfy their needs by the diet. For a large portion of humankind though, it is very tough to sustain the daily intake on sufficient levels. One striking idea to boost folate intake is to employ biotechnology to improve the concentration of ordinary folates in food-as opposed to supplement food by using man-made folates or use supplementation by tablets.

Baker's yeast, *Saccharomyces cerevisiae*, has been found to generally contain a relatively high amount of folate per weight (Witthöft *et al.,* 1999). Seyoum and Selhub (1998) described a total folate content of 24.5 µg/g of dry matter of yeast while Patring and Jastrebova (2007) reported 35.2 µg/g. Folates from yeast obviously add to the finishing folate content in yeast fermented foodstuffs, such as bread (Kariluoto *et al.,* 2004; Gujska and Majewska, 2005) and kefir (Drewek and Czarnocka-Roczniakowa, 1986). In wheat bread folate levels were improved 2.5 times when using yeast, in place of baking powder, as leavening agent (Kariluoto *et al.,* 2004).

Experimental structure on fermentation

Henry and Saini (1989) described that the most significant carbohydrates from flour influencing the loaf volume are glucose, fructose and sucrose. The arrangement in which these different carbohydrates are fermented by *Saccharomyces cerevisiae* is not at random, but rather is based on a specific pecking order, glucose being the preferred sugar. It is considered that glucose decreases the uptake of fructose because both sugars are

imported by the same carriers, which have a greater empathy for glucose than for fructose (Verstrepen *et al.,* 2004).

Of the above mentioned carbohydrates, sucrose is changed almost right away to glucose and fructose, due to the effective invertase of yeast (Sahlsrtom *et al.,* 2003). When the concentration of glucose and fructose is elevated enough, the maltose concentration in dough is also mounted due to amylase, a starch debasing enzyme in flour, which is continuously generating new glucose and maltose in flour starch. When glucose and fructose are ended, the maltose concentration begins to lessen, making difficult for yeast cells to hydrolyze since they do not have the essential enzymatic tools in time, working methods and techniques such as thin layer chromatography (Sasano *et al.,* 2012).

Mathematical modeling of dough behavior during yeast fermentation

Bread making is fundamentally a temperature-dependent two step progression, consisting of fermentation, in which CO_2 production linked with yeast activity is manifested in porous dough structure with the development of dough volume during baking where yeast activity is ended and the bread structure is finalized. During baking, the inside temperature reaches 100 °C and the volume fraction of bread reaches a final value between 0.8 and 0.9 (Shehzad *et al.,* 2010; Shehzad *et al.,* 2011), while gluten cross-links and starch granules are disrupted (Franci and Igore, 2011).

The concluding bread structure depends on dough ingredients, yeast activity, fermentation temperature and gas bubble formation. So far, the bread making has been studied at different scales by various imaging modalities, such as flatbed scanning and conventional photography (Lassoued *et al.,* 2007), as well as by more highly developed high-resolution techniques, e.g., scanning electron microscopy (Hayman *et al.,* 1998), X-ray computed tomography (Babin *et al.,* 2006; Turbin-Orger *et al.,* 2012) and magnetic resonance imaging (MRI). The effect of yeast during fermentation of wheat flour dough was also studied at macro scale (Shehzad *et al.,* 2010). Among all these techniques, both at macro and micro scales, MRI has numerous advantages due to its non invasiveness, precise moisture content determination and a comparatively high spatial resolution. For example, Ishida *et al.,* (2001) employed MRI to analyze differences in architecture between breads made from fresh and frozen dough. To improve an image contrast and to shorten relaxation times, they soaked bread samples in acetone with added paramagnetic substances prior to imaging.

One of the first MRI experiments with dynamic imaging of baking was done by (Hong *et al.,* 1996) who introduced a specially designed MRI oven, constructed from nonmagnetic materials. This was used to learn cookie baking in a low field MRI scanner (0.6 T). Another similar experiment was done by (Wagner *et al.,* 2008) who used a spacious MRI oven well-matched with a low-field MRI scanner (0.2 T) to monitor bread loaf fermentation and baking. (De Guio *et al.,* 2009) used vulnerability effects in low-field MRI (0.2 T) to study the growth of pores in different dough (yeasted and non-yeasted) during fermentation. Results of the study showed that pores have a Gaussian-like size (radii) distribution with a gradual increasing average size that is associated with the dough rise during fermentation.

All the above-presented low-field MRI experiments have a good temporal resolution and image quality; however, they are lacking in spatial resolution. The resolution problem can be overcome by the use of high-field MRI scanners that permit magnetic resonance microscopy (MRM) experiments. One such experiment was done by (van Duynhoven *et al.,* 2003) who dynamically imaged dough fermentation using a 4.7-T MRI scanner with a spatial resolution of 0.27×0.27×3 mm and a temporal resolution of 2 min. Advanced magnetic field of 9.4 T was employed in experiments done by (Bonny *et al.,* 2004) who imaged the same process at a resolution of $0.12\times0.12\times0.5$ mm^3, but with a lower temporal resolution of 8.5 min. A similar study was completed also using 3D MR imaging with an isotropic resolution below 100 μm (Takano *et al.,* 2002).

All these high-field MRI studies were constrained by constant sample volume changes, so that the optimal imaging parameters were selected as the best cooperate between the spatial resolution and the temporal resolution, i.e., too low a temporal resolution would result in motional blurring. Image processing routines are a powerful device in analysis of dough texture properties. Standard image processing techniques (thresholding, particle counting, area and volume measurements) are inadequate to extort all available information on dough fermentation and baking (Ishida *et al.,* 2001). Therefore, advanced image processing techniques, as for example mathematical morphology routines (dilation, erosion, closing, opening, etc.), are often used in addition to the standard ones (Rouille *et al.,* 2005).

Dough is a multiphase and multi-component system largely composed of proteins, lipids, carbohydrates, water and air. The dough ingredients, as well as the processing conditions, determine the macroscopic structure of baked foodstuffs which, in turn, is responsible for their appearance, texture, taste and stability. To build up this structure, the ingredients are mixed and kneaded, the dough leavened and baked. Enormous structural changes take place during the bread making methods (Autio and Laurikainen, 1997). During mixing, the ingredients are transformed into a visco-elastic material as a result of the formation of a three-dimensional protein network, in which starch granules are consistently detached. During kneading, air bubbles are built-in in the dough and they are assumed to be the early nuclei of the gas bubble, which will build up during the

succeeding stages. During leavening, the metabolism of yeasts chemically transforms assimilable carbohydrates into carbon dioxide and ethyl alcohol as the principal finished products. As a related amount of alcohol forms, which is water-miscible, it influences the colloidal nature of the wheat proteins and changes the interfacial tension within the dough. In addition, carbon dioxide, which partly dissolves in the aqueous phase of the dough, migrates toward the initial nuclei of the air bubbles formed during kneading causing their growth. The growth of gas cells depends on the cell size and the dough composition. Certain ingredients are known to exert a stabilizing influence and retard coalescence (Gan, Ellis, and Schofield, 1995). It is important to distinguish between gas production and gas retention in fermented doughs (Cauvain, 2001). The first factor is controlled by the yeast performance and the last one depends on the bubble characteristics. The desirable loaf volume of yeast-fermented products is achieved only if the dough provides a favorable environment for yeast growth and gas generation and, at the same time, possesses a gluten matrix capable of maximum gas retention (Sahlstro¨m, Park, and Shelton, 2004). The latter attribute is most conveniently determined by measuring the volume increase of fermenting dough, whereas gas production can be estimated by any of the several available procedures such as the oven rise recorder (Marek and Bushuk, 1967), alveograph method (Approved Method 54-40, AACC, 2000) and pressure meter methods (Bailey, 1939; Malloch, 1939). Yeast-fermented doughs are difficult to study, because they are very complex, and the dimensions and physical properties of the dough change with time (Bloksma, 1990; Szczesniak, 1988). Furthermore invasive, continuous measurements on dough are generally not adequate as they may provoke dough collapse. The choice of the most appropriate analytical procedure is thus crucial for the full comprehension of the underlying mechanisms of leavening. From a structural point of view bread dough is an elastic foam and leavening is a process very similar to the expansion of a pseudoplastic foam, in which initial germs (yeast) are quasi-homogeneously distributed into the dough volume. Little is known about the physical processes governing foam formation. Some of the main issues are the lack of robust test methods to quantify their behavior, concerns about the reliability of the data, variability in the material properties and the need to relate structure to behavior (Lim and Barigou, 2004). There is a real need for robust quantitative methods for characterizing the structure of these materials, so that intrinsic relationships between structure and properties can be developed. Image analysis is potentially a non-intrusive, objective method for measurement and comparison of the structure of food foams that will allow quality control and process optimization (Cilliers and Sadr-kazemi, 1999). The most apparent physical change related to the development of fermentation in the dough is the increase in its volume (Pyler, 1988). Although, an extensive the literature exists dealing with the control of the leavening process (De Cindio and Correra, 1995; Dixon and Kell, 1989; Pinter, 1988) and mathematical models and equations for expression of microbial growth in food (Fan, Yingying, Qian, and Gu, 2004; Fujikawa, Kai, and Morozumi, 2004; Vadasz, Vadasz, Abashar, and Gupthar, 2001), the description of such a process will always be a rough simplification of reality, since detailed picture of the various biological and physical phenomena responsible for bubbles growth during the leavening process are still difficult to model. Fermentation involves biochemical, rheological and thermodynamic phenomena, which are nonlinear distributed-parameter processes. Growth curves are generally of sigmoid shape with a first stage in which the specific growth rate starting from zero slowly increases for a period of time known as lag time. After this period, a fast increasing growth rate phase follows in which a maximum rate value is achieved at the inflection point (Shehzad *et al.,* 2010). Finally, a plateau is reached in a final phase in which the rate decreases and eventually became zero. These kinds of sigmoid curves can be fitted by different mathematical functions, such as monomolecular, von Bertalanffy, Gompertz and logistic (McCallum and Dixon, 1990). A major development in the analysis of growth curves has been the generalization of these sigmoid growths to a single function, i.e. the Gompertz function (Zwietering, Jongenburger, Rombouts, and van't Riet, 1990). Since that, the Gompertz ($y = \ln x(t)$) model has become the standard growth model in predictive microbiology for modeling growth of pathogens and spoilage bacteria in food (Whiting and Buchanan, 1994). The effect of yeast on dough volume during fermentation was modeled by different researchers using Gompertz equation (Romano *et al.,* 2007; Shehzad *et al.,* 2010; Kansou *et al.,* 2012), thus providing very useful information about various aspects of fermentation, especially evolution of dough volume during yeast fermentation.

Conclusion

Yeast plays a vital role in dough expansion during fermentation due to CO_2 production along with development of flavor and alcohol synthesis. Although various studies indicated its importance during the fermentation process but its role was not thoroughly discussed which needed to be addressed. In its natural, fresh form, yeast is considered to act as catalyst for breakdown of sugars due to fermentation process. Different types and forms of yeast develop different flavors and may give rise to dough volume depending upon amount of gas produced within the dough as a result of yeast action on sugars. Although, dough behavior during fermentation can be presented in form of sigmoidal curves using different mathematical models and has been studied by various researchers but the effect of different types and forms of yeasts on dough behavior

during fermentation needs further study and can elucidate useful results for breadmaking industry.

REFERENCES

1. American Association of Cereal Chemists. 2000. Approved Methods of AACC, 10th edition. Methods 08–01, 10–10B, 22–11, 44–15A, 46–10, 54–40, and 56–81B. The Association, St. Paul, Minnesota.
2. Ando, A., F. Tanaka, Y.Murata, H.Takagi and J. Shima. 2006. Identification and classification of genes required for tolerance to high-sucrose stress revealed by genome-wide screening of Saccharomyces cerevisiae. FEMS Yeast Research 6, 249–267.
3. Attfield, P.V. 1997. Stress tolerance: the key to effective strains of industrial baker's yeast. NatureBiotechnology15,1351_1357.Availablefo rm:http://www.eufic.org/gb/food/pag/food22/food222.htm [accessed 1 Oct 2012]
4. Autio, K., & Laurikainen, T. 1997. Relationships between flour/dough microstructure and dough handling and baking properties. Trends Food Science and Technology, 8, 181–185.
5. Babin, P., G. Della Valle, H. Chiron, P. Cloetens, J. Hoszowska, P. Pernot, et al. 2006. Fast X-ray tomography analysis of bubble growth and foam setting during breadmaking. J Cereal Sci 43 (3):393–7.
6. Bailey, C. H. 1939. Measuring fermentation rate and gas losses in dough. Cereal Chemistry, 16, 665–670.
7. Bauer, S.P.A. 2005. Bauer Products, Useful Information [online]. Available from: http://www.bauerspa.it/eng/prodotti/utili.htm [accessed 02Oct 2012].
8. Berry, R.J., Z. Li, J.D. Erickson, S. Li, C.A. Moore, H. Wang, J. Mulinare, P. Zhao, L.Y.C. Wong, J. Gindler, S.X. Hong and A. Correa. 1999. Prevention of neural-tube defectswith folic acid in China. New England Journal of Medicine 341, 1485–1490.
9. Bloksma, A. H. 1990. Dough structure, dough rheology and baking quality. Cereal Food World, 35, 237–244.
10. Bonny, J.M., J. Rouille, G. Della Valle, M.F. Devaux, J.P. Douliez and J.P. Renou. 2004. Dynamic magnetic resonance microscopy of flour dough fermentation. Magnetic Resonance Imaging 22(3):395–401.
11. Brouwer, I.A., M. Van Dusseldorp, C.M.G.Thomas, M. Duran, J.G.A.J. Hautvast, , T.K.A.B. Eskes, R.P.M. Steegers-Theunissen. 1999. Low-dose folic acid supplementation decreases plasma homocysteine concentrations: a randomized trial. American Journal of Clinical Nutrition 69, 99–104.
12. Cauvain, S. P. 2001. Breadmaking. In Gavin Owens (Ed.), Cereals processing technology. Cambridge England: CRC Press (chap. 10).
13. Choi, S.W. and J.B.Mason. 2000. Folate and carcinogenesis: an integrated scheme. Journal of Nutrition 130, 129–132.
14. Cilliers, J. J. L., and N. Sadr-kazemi. 1999. Image analysis of food foams. Bubbles in foods (pp. 245–251). St. Paul, Minnesota: Eagan Press.
15. Cronwright, G.R., J.M. Rohwer and B.A. Prior. 2002. Metabolic control analysis of glycerol synthesis in Saccharomyces cerevisiae. Applied and Environmental Microbiology 68, 4448–4456.
16. De Cindio, B. and S.Correra. 1995. Mathematical modelling of leavened cereal goods. Journal of Food Engineering, 24, 379–403.
17. De Guio, F, M. Musse, H. Benoit-Cattin, T. Lucas and A. Davenel. 2009. Magnetic resonance imaging method based on magnetic susceptibility effects to estimate bubble size in alveolar products: application to bread dough during proving. Magn Reson Imaging 27(4):577–85.
18. De Virgilio, C., T. Hottiger, J. Dominguez, T. Boller, and A.Wiemken. 1994. The role of trehalose synthesis for the acquisition of thermotolerance in yeast. I. Genetic evidence that trehalose is a thermoprotectant. European Journal of Biochemistry/FEBS 219, 179–186.
19. Dengate, S. and A. Ruben. 2002. Controlled trial of cumulative behavioural effects of a common bread preservative. Journal of Paediatrics and Child Health 38, 373–376.
20. Dixon, N., and D. Kell. 1989. The control and measurement of CO2 during fermentation. Journal of Microbiological Methods, 10(3), 55–176.
21. Drewek, Z. and B.Czarnocka-Roczniakowa. 1986. Microbiological processes in folacin synthesis in kefir. Acta Biochimica Polonica 12, 39–45.
22. Fan, Y., W. Yingying, P. Qian and J. Gu. 2004. Optimization of phthalic acid batch biodegradation and the use of modified Richards model for modelling degradation. International Biodeterioration and Biodegradation, 53, 57–63.
23. Franci B. and S. Igor. 2011. Continuous monitoring of dough fermentation and bread baking by magnetic resonance microscopy, Magnetic Resonance Imaging, 29:434–442.
24. Fujikawa, H., A. Kai and S. Morozumi. 2004. A new logistic model for Escherichia coli growth

at constant and dynamic temperatures. Food Microbiology, 21, 501–509.
25. Gan, Z., P.R. Ellis and J. D. Schofield. 1995. Mini review: gas cell stabisation and gas retention in wheat bread dough. Journal of Cereal Science, 21, 215–230.
26. Hayman, D., R.C. Hoseney and J.M. Faubion. 1998. Bread crumb grain development during baking. Cereal Chemistry 75(5):577–80.
27. Henry, R.J. and H.S. Saini. 1989. Characterization of cereal sugars and oligosaccharides, Cereal Chemistry 66(5): 362-365.
28. Hino, A., K. Mihara, K.Nakashima and H.Takano. 1990. Trehalose levels and survival ratio of freeze-tolerant versus freeze-sensitive yeasts. Applied and Environmental Microbiology 56, 1386–1391.
29. Hirasawa, T., Y. Nakakura, K.Yoshikawa, K. Ashitani, K. Nagahisa, C. Furusawa, Y. Katakura, H. Shimizu and S. Shioya. 2006. Comparative analysis of transcriptional responses to saline stress in the laboratory and brewing strains of Saccharomyces cerevisiae with DNA microarray. Applied Microbiology and Biotechnology 70, 346–357.
30. Hong, S.W., Z.Y. Yan, M.S. Otterburn and M.J. McCarthy. 1996. Magnetic resonance imaging (MRI) of a cookie in comparison with time-lapse photographic analysis (TLPA) during baking process. Magn Reson Imaging 14(7–8):923–7.
31. Hutkins, R.W. 2006. Bread fermentation, in Microbiology and Technology of Fermented Foods, ed. By Blackwell Publishing, 261-299.
32. Ishida N, Takano H, Naito S, Isobe S, Uemura K, Haishi T, et al. Architecture of baked breads depicted by a magnetic resonance imaging. Magn Reson Imaging 2001;19(6):867–74.
33. Kaino, T. and H. Takagi, , 2008. Gene expression profiles and intracellular contents of stress protectants in Saccharomyces cerevisiae under ethanol and sorbitol stresses. Applied Microbiology and Biotechnology 79, 273–283.
34. Kaino, T., T. Tateiwa, S. Mizukami-Murata, J. Shima, H.Takagi. 2008. Self-cloning baker's yeasts that accumulate proline enhance freeze tolerance in doughs. Applied and Environmental Microbiology 74, 5845–5849.
35. Kansou, K., H. Chiron, G. Della Valle, A. Ndiaye, P. Roussel and A. Shehzad. 2012. Modelling wheat flour dough proofing behaviour: Effects of mixing conditions on porosity and stability. Food and Bioprocess Technology. DOI: 10.1007/s11947-012-0854-1.
36. Kariluoto, S., Vahteristo, L., Salovaara, H., Katina, K., Liukkonen, K.H., Piironen, V., 2004. Effect of baking method and fermentation on folate content of rye and wheat breads. Cereal Chemistry 81, 134–139.
37. Kramer, J.M. and R.J.Gilbert. 1989. Bacillus cereus and other Bacillus species. In: Doyle, M.P. (Ed.), Foodborne bacterial pathogens. Marcel Dekker, New York, pp. 22–70.
38. Kristiansen, B. 1994. Integrated design of a fermentation plant : the production of baker's yeast.
39. Lallemand Inc. 2001. Yeast Production, Lallemand Baking Update, Volume 1/Number 9 [online].Availablefrom:http://www.lallemand.com/BakerYeastNA/eng/PDFs/LBU%20PDF%20FILES/1_9YPROD.PDF[accessed 02Oct 2012].
40. Lassoued, N., P. Babin, G. Della Valle, M.F. Devaux and A.L. Reguerre. 2007. Granulometry of bread crumb grain: contributions of 2D and 3D image analysis at different scale. Food Research International 40(8):1087–97.
41. Lim, K.S. and M. Barigou. 2004. X-Ray micro-computed tomography of aerated cellular food products. In: International Conference on Engineering and Food- ICEF9, 7–11 March.
42. Lucock, M. 2000. Folic acid: nutritional biochemistry, molecular biology, and role in disease processes. Molecular Genetics and Metabolism 71, 121–138.
43. Madigan M.T., J.M. Martinko and J. Parker. 2003. Brock Biology of Microorganisms, 10th Edition, Pearson Education Inc.
44. Malloch, J. G. 1939. A convenient apparatus for gas production determinations by the Blish method. Cereal Chemistry, 16, 178–182.
45. Marek, C., and W. Bushuk. 1967. Study of gas production and retention in doughs with a modified Brabender oven-rise recorder. Cereal Chemistry, 44, 300–307.
46. Marin, S., M.E. Guynot, P.Neira, M. Bernado, V. Sanchis and A.J. Ramos. 2002. Risk assessment of the use of sub-optimal levels of weak-acid preservatives in the control of mould growth on bakery products. International Journal of Food Microbiology 79, 203–211.
47. Matsuura, K. and H.Takagi. 2005. Vacuolar functions are involved in stress-protective effect of intracellular proline in Saccharomyces cerevisiae. Journal of Bioscience and Bioengineering 100, 538–544.
48. McCallum, D.A., and P.M. Dixon. 1990. Reducing bias in estimates of Richards growth function shape parameter. Growth, Development and Aging, 54, 135–141.
49. Morita, Y., S. Nakamori and H.Takagi. 2002. Effect of proline and arginine metabolism on freezing stress of Saccharomyces cerevisiae. Journal of Bioscience and Bioengineering 94, 390–394.

50. O'Shea, D. 2005. BE401 Industrial Bioprocessing – Module Notes [online] Available from: and Feed, 2nd Edition, Vol. 9
51. Patring, J.D.M. and J.A. Jastrebova, , 2007. Application of liquid chromatography-electrospray ionisation mass spectrometry for determination of dietary folates: effects of buffer nature and mobile phase composition on sensitivity and selectivity. Journal of Chromatography A 1143, 72–82.
52. Pattison, T.L., D. Lindsay and A. von Holy. 2004. Natural antimicrobials as potential replacements for calcium propionate in bread. South African Journal of Science 100, 342–348.
53. Pinter, J. 1988. In line control in the baking industry based on instrumental measurements. Elelmezesi Ipar, 42(8), 294–299.
54. Pyler, E. J. 1988. Dough fermentation (3rd edition. Baking science and technology, pp. 625–659). Kansans City, Missouri: Sosland Publishing Company.
55. Rehm, H.J., G. Reed, A. Puhler and P. Stadler . 1995. Biotechnology – Enzymes, Biomass, Food
56. Romano, A., G.Toraldo, S. Cavella and P. Masi. 2007. Description of leavening of bread dough with mathematical modelling. Journal of Food Engineering 83, 142−148.
57. Rosenkvist, H., and Å. Hansen. 1995. Contamination profiles and characterization of Bacillus species in wheat bread and raw materials for bread production. International Journal of Food Microbiology 26, 353–363.
58. Rosenkvist, H. and Å. Hansen. 1995. Contamination profiles and characterization of Bacillus species in wheat bread and raw materials for bread production. International Journal of Food Microbiology 26, 353–363.
59. Rouille, J., J.M. Bonny, G. Della Valle, A.F. Devaux and J.P. Renou. 2005. Effect of flour minor components on bubble growth in bread dough during proofing assessed by magnetic resonance imaging. Journal of Agricultural Food Chemistry 53(10):3986–94.
60. Sahlstro¨m, S., W. Park, and D. R. Shelton. 2004. Factors influencing yeast fermentation and the effect of LMW sugars and yeast fermentation on hearth bread quality. Cereal Chemistry, 81(3), 328–335.
61. Sahlstrom, S., W. Park and D.R. Shelton. 2003. Factors influencing yeast fermentation and the effect of LMW sugars and yeast fermentation on hearth bread quality, Cereal Chemistry, 81 (3): 328–335.
62. Sasano Y., Y. Haitani, I. Ohtsu , J. Shima and H. Takagi. 2012. Proline accumulation in baker's yeast enhances high-sucrose stress tolerance and fermentation ability in sweet dough. International Journal of Food Microbiology 152, 40–43.
63. Seshadri, S., A. Beiser, J. Selhub, P.F. Jacques, I.H. Rosenberg, R.B. D'Agostino, P.W.F. Wilson and P.A.Wolf. 2002. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. New England Journal of Medicine 346, 476–483.
64. Seyoum, E. and J.Selhub. 1998. Properties of food folates determined by stability and susceptibility to intestinal pteroylpolyglutamate hydrolase action. Journal of Nutrition 128, 1956–1960.
65. Shehzad, A., H. Chiron, G. Della Valle, K. Kansou, A. Ndiaye, A- L. Reguerre. 2010. Porosity and stability of bread dough during proofing determined by video image analysis for different compositions and mixing conditions. Food Research International, 43: 1999-2005.
66. Shehzad, A., L. Chaunier, H. Chiron, G. Della Valle, M. Ducasse, D. Lourdin, A- L. Reguerre and L. Saulnier. 2011. Processing doughs for bread with improved nutritional properties to incorporation of dietary fibers. Pakistan Journal of Food Sciences, 21(1-4): 56-66.
67. Shima, J., A. Hino, C. Yamada-Iyo, Y. Suzuki, R. Nakajima, H.Watanabe, K.Mori and H. Takano. 1999. Stress tolerance in doughs of Saccharomyces cerevisiae trehalase mutants derived from commercial baker's yeast. Applied and Environmental Microbiology 65, 2841–2846.
68. Shima, J., H. Takagi. 2009. Stress-tolerance of baker's-yeast (Saccharomyces cerevisiae) cells: stress-protective molecules and genes involved in stress tolerance. Biotechnology and Applied Biochemistry 53, 155–164.
69. Sorokulova, I.B., O.N. Reva, V.V. Smirnov, I.V. Pinchuk, S.V. Lapa and M.C. Urdaci. 2003. Genetic diversity and involvement in bread spoilage of Bacillus strains isolated from flour and ropy bread. Letters in Applied Microbiology 37, 169–173.
70. Spicher, G. 1983. Baked goods. In: Rehm, H.J., Reed, G. (Eds.), Biotechnology. Verlag Chemie, Weinheim, pp. 1–80.
71. Szczesniak, A. S. 1988. The meaning of textural characteristics. Journal of Texture Studies, 19, 51–59.
72. Takagi, H., F. Iwamoto and S.Nakamori. 1997. Isolation of freeze-tolerant laboratory strains of Saccharomyces cerevisiae from proline-analogue-resistant mutants. Applied Microbiology and Biotechnology 47, 405–411.
73. Takagi, H., K. Sakai, K. Morida and S. Nakamori. 2000. Proline accumulation by

mutation or disruption of the proline oxidase gene improves resistance to freezing and desiccation stresses in Saccharomyces cerevisiae. FEMS Microbiology Letters 184, 103–108.

74. Takagi, H., M. Takaoka, A.Kawaguchi and Y.Kubo. 2005. Effect of L-proline on sake brewing and ethanol stress in Saccharomyces cerevisiae. Applied and Environmental Microbiology 71, 8656–8662.
75. Takagi, H. 2008. Proline as a stress protectant in yeast: physiological functions,metabolic regulations, and biotechnological applications. Applied Microbiology and Biotechnology 81, 211–223.
76. Takano, H., S. Naito, N. Ishida, M. Koizumi and H. Kano. 2002. Fermentation process and grain structure of baked breads from frozen dough using freeze-tolerant yeasts. Journal of Food Science 67(7):2725–33.
77. Tanaka, F., A. Ando, T.Nakamura, H.Takagi and J. Shima. 2006. Functional genomic analysis of commercial baker's yeast during initial stages of model dough-fermentation. Food Microbiology 23, 717–728.
78. Tanaka-Tsuno, F., S. Mizukami-Murata, Y. Murata, T. Nakamura, A.Ando, H. Takagi and J. Shima. 2007. Functional genomics of commercial baker's yeasts that have different abilities for sugar utilization and high-sucrose tolerance under different sugar conditions. Yeast (Chichester, England) 24, 901–911.
79. Team Minn-Dak Yeast, 2005. Bakers Yeast Production [online]. Available from: http://www.dakotayeast.com/yeast_production.html. [accessed 17Oct 2005].
80. Terao, Y., S. Nakamori and H. Takagi. 2003. Gene dosage effect of L-proline biosynthetic enzymes on L-proline accumulation and freeze tolerance in Saccharomyces cerevisiae. Applied and Environmental Microbiology 69, 6527–6532.
81. The Artisan, The Yeast Treatise, 2002, Yeast Production - General Discussion [online]. Available from: http://www.theartisan.net/yeast_treatise_frameset.htm [accessed on 4 Oct 2010]
82. Turbin-Orgera, A., E. Boller, L. Chaunier, H. Chiron, G. Della Valle and A.-L. Réguerre. 2012. Kinetics of bubble growth in wheat flour dough during proofing studied by computed X-ray micro-tomography. Journal of Cereal Science. In Press.
83. Vadasz, A. S., P. Vadasz, M. E. Abashar and A. S. Gupthar. 2001. Recovery of an oscillatory mode of batch yeast growth in water for a pure culture. International Journal of Food Microbiology 71, 219–234.
84. van Duynhoven, J.P.M., G.M.P. van Kempen, R. van Sluis, B. Rieger, P. Weegels, L.J. Vliet, et al. 2003. Quantitative assessment of gas cell development during the proofing of dough by magnetic resonance imaging and image analysis. Cereal Chemistry 8(4):390–5.
85. Van Hoek, P., A. Aristidou, J. Hahn and A. Patist. 2003. Fermentation Goes Large-Scale [online].
86. Verbruggen, N. And C. Hermans. 2008. Proline accumulation in plants: a review. Amino Acids 35, 753–759.
87. Verstrepen, K.J., D. Iserentant, P.Malcorps, G.Derdelinckx, P.Van Dijck, J.Winderickx, and I.S. Pretorius. 2004. Glucose and sucrose: hazardous fast-food for industrial yeast?. Trends in Biotechnology 22(10): 531–537.
88. Viljoen, C.R. and A. von Holy. 1997. Microbial populations associated with commercial bread production. Journal of Basic Microbiology 37, 439–444.
89. Voysey, P.A. and J.C. Hammond. 1993. Reduced-additive bread-making technology. In: Smith, J. (Ed.), Technology of reduced-additive foods. BlackieAcademic& Professional, London, pp. 80–94.
90. Wagner, M.J., M. Loubat, A. Sommier, D. Le Ray, G. Collewet, B. Broyart, et al. 2008. MRI study of bread baking: experimental device and MRI signal analysis. International Journal of Food Scienve and Technol 43(6):1129–39.
91. Wald, N.J., A.K. Hackshaw, R. Stone, N.A. Sourial. 1996. Blood folic acid and vitamin B12 in relation to neural tube defects. British Journal of Obstetrics and Gynaecology 103, 319–324. Weinheim, New York.
92. Whiting, R. C. and R. L. Buchanan. 1994. Scientific status summary: microbial modeling. Food Technology 48, 113–120.
93. Wickramasinghe, S.N. 2006. Diagnosis of megaloblastic anaemias. Blood Reviews 20, 299–318.
94. Witthöft, C.M., K. Arkbåge , M. Johansson , E. Lundin , G.Berglund, J.X. Zhang, H. Lennernås, J.R. Dainty. 2006. Folate absorption from folate-fortified and processed foods using a human ileostomy model. British Journal of Nutrition 95, 181–187.
95. Zwietering, M. H., I. Jongenburger , F. M. Rombouts and K. van't Riet. 1990. Modeling of the bacterial growth curve. Applied and Environmental Microbiology, 56, 1875–1881.