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CONTENT

Short Communication

STUDIES ON OBTAINING OF DRINKING WATER QUALITY IN A DRINKING WATER TREATMENT PLANT

Elena Cical[1,2], Mircea Mecea,[1,2] Gheorghe Vâtcă[2], Cristina Mihali[2], Narcis Duteanu[3]

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Abstract

The paper presents studies regarding the water quality from the accumulation lake, characterised by higher turbidity in the studied period, and also the water treatment possibilities with new coagulation reagents (aluminium bases poly chlorines) and alkalisation, comparative with aluminium sulphate and calcium hydroxide, following the efficiency and inefficiency of those in the colloidal systems destabilisation and zeta potential reduction.

Keywords: drinking water, aluminium sulphate, base poly chlorine of aluminium

1. Introduction

Water is the most important food, can not be replaced. The human can live in extremis without water for other uses, but not without the drinking water. He resist a long time without food but without water only a short time. From this reasons for the human the most important water was, is and will be the drinking water. [1, 2]

In natural conditions, water is never found in pure state, in it being always any quantities of dissolved chemical substances or in suspension. The suspension removal is an obligation of the water treatment stations, which by tackled treatment procedures, realize a technological flux which assure the drinking water quality in the limits anticipated by the legislation.

In the treatment water, the coagulation process is used for favor the aggregation, to transform a stable suspension in one instable. Through coagulation the particles, which staid in lake months or years, can be aggregated in less an hour. The coagulation process must be lead so that to can change and control the aggregation state nature of the particles.

Aluminium sulphate with the chemical formula $Al_2(SO_4)_2 \cdot 18\ H_2O$ and an Al content of 8.1% Al, is an incontestable efficient coagulant, in most of the cases his results are mediocre in the case of water with low temperatures, low turbidity and small alkalinities. The hydrolyse velocity is low, the quantity of sulphate aluminium is dosed and the residual concentration of aluminium is higher, so the coagulation process efficiency is low [3,6].

For the improving of the coagulation process was used pre hydrolysed and pre polymerised coagulants. These are partial hydrolysed and have the next formula: $Al_a(OH)_b(X)_c$. [4]

Base poly chlorine of aluminium with the chemical formula $Al_n(OH)_m(Cl)_{3n-m}$ is a coagulation reagent partial hydrolysed, based on aluminium chlorine [5]. Is an acid product under liquid form (density = 1.27 kg/dm^3 and an Al content of 10%), can be does in installation in the diluted solution.

The main sources of the Baia Mare municipal supply with water is the accumulation lake Strimtori Firiza. In general the water quality is good with the exception of 4-5 months per year, when

because of the abundant rains is obtained higher turbidity of the raw water. Practical trough the studied technological flux is whished an improving of the drinking water quality obtained in the treatment station in accordance with the legislation requirements.

2. Materials and methods

In the present paper was studied comparative the efficient use of aluminium sulphate and aluminium base poly chlorine in the coagulation process of the suspensions from water. In the view of optimum pH establish for the both coagulation reagent was used the calcium hydroxide. The practical studies were made on the station "Micro plant" during the technological sample. The optimum coagulation dose for the both reagents were determinate experimental in laboratory on water with different turbidity using the Jar-test method in the next conditions: 2 minutes of fast stirring (140 rot/min) and 15 minutes of slow stirring (40 rot/min) and 30 minutes of settling. Function of optimum coagulation doses obtained in laboratory for aluminium sulphate and aluminium base poly chlorine, was passed at their applying in the technological flux. Practical was studied a technical month of treatment using aluminium sulphate and calcium hydroxide and a technical month of treatment using aluminium base poly chlorine and calcium hydroxide.

The water turbidity was determinate using a turbidity meter WTW 350 IR. The water pH was determinate using a Hanna pH-meter. The water oxidability and hardness were determinate using the volumetrically chemical methods. The residual aluminium, nitrates, nitrites were determinate using an atomic absorption spectrophotometer Hach DR 2000. All the determination methods of the parameters are in accordance with the legislation requirements regarding the quality of drinking water.

3. Results and discussions

The technological flux of the station "Micro plant" contain the next stages: catching of the raw water, blending room, reaction room combined with lamellar clarifying tank, water filtration trough filters under pressure, water disinfection with sodium hypochlorite and drinking water storage.

a) Characterisation of the water sources

In Table 1 are presented the main parameters of the raw water in the studied period:

Table 1. *The parameters of the raw water*

No. crt.	Parameter	Value	
		Minim	Maxim
1.	Turbidity, NTU	4,00	16,0
2.	pH	6.70	6.80
3.	Alkalinity, mval/L	0.50	0.50
4.	Oxidability, mg/L O_2	1.97	2.76
5.	Hardness, D	1.68	1.79
6.	Aluminium, mg/L	0.008	0.028
7.	Nitrites, mg/L	0.000	0.009
8.	Nitrates, mg/L	0.325	0.450

b) The coagulation process efficiency using aluminium sulphate and calcium hydroxide

In the frame of the made studies was followed the coagulation process efficiency using aluminium sulphate with calcium hydroxide. This treatment technique correspond to an optimum pH = 7.0.

In Figure 1 are presented the optimum doses of aluminium and calcium hydroxide obtained experimental in

laboratory on raw water with different turbidity and which were applied practical in the water treatment process. The main parameter studied during a month was the turbidity of the drinking water obtained in the station "Micro plant". In Figure 2 is presented the turbidity of the obtained drinking water.

From the experimental data can be observed that with the increasing of the raw water turbidity increases considerable the quantity needed of the aluminium sulphate. The quantity of calcium hydroxide represent almost 70 % from the added quantity of aluminium sulphate. From the data presented in Figure 2 can be observed that the turbidity of the treated water don't correspond, the values being much higher over the maximum concentration admitted by the drinkable water quality legislation. In conclusion this technique does not present efficiency on the coagulation process for the studied technological flux.

c) The coagulation process efficiency using aluminium base poly chlorine and calcium hydroxide

In the frame of the made studies was followed the coagulation process efficiency using aluminium base poly chlorine (PAC) and calcium hydroxide. This treatment technique correspond to an optimum pH = 7.8. In Figure 3 are presented the optimum doses of the aluminium base poly chlorine and calcium hydroxide experimental obtained in laboratory on raw waters with different turbidity and which were applied in the water treatment process. The dose of calcium hydroxide needed to increase the alkalinity of raw water corresponds to the value of 15 mg/L and is constant indifferently by the turbidity of raw water. The main parameter studied during one month was the drinkable water turbidity obtained in the station "Micro plant". In Figure 2 are presented the turbidity of the obtained drinking water.

From the presented data in Figure 3 can be observed that with the increasing of the raw water turbidity increase the needed quantity of PAC, this increasing is higher until a turbidity of 20 NTU, after this turbidity the quantity of PAC present a slow increasing. These quantities are lower than the needed quantities of aluminium sulphate or the same turbidity of water. From the experimental data presented in Figure 4 can be observed that trough this technique is obtained turbidity of the treated water lower than the maximum concentration admitted by the water quality legislation (turbidity-CMA < 5 UNT).In conclusion this treatment technique presents efficiency on the coagulation process for the studied technological flux.

In the view of obtained drinking quality assurance, were analysed the main parameters: residual aluminium, oxidability, nitrates and nitrites.

The use of the coagulation reagents based on aluminium leads in most of the cases to higher concentrations of aluminium in the treated water than in the raw water; practical it's wanted a reduction of that aluminium content from the treated water. In Figure 5 is presented the residual aluminium dependences versus days. The organic materials from water influence the chose of the coagulant dose in the same way as the inorganic particles. In Figure 6 are presented the oxidability dependence versus days.

The nitrates present in drinkable water over the admitted limit, determine negative effects on the human health. Noxious are in fact the nitrites which results from nitrates in special conditions, in organism but also abiogenesis in galvanised pipes, where the nitrates are reduced at nitrites generating a secondary toxicity of the nitrates. In Figures 7 and 8 are presented the nitrates and nitrites dependence versus days.

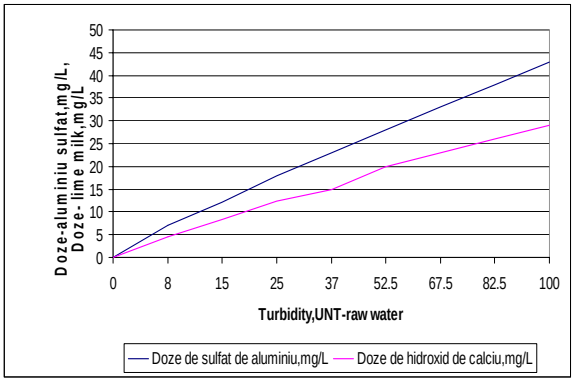

Figure 1. The optimum doses of $Al_2(SO_4)_3$ and calcium hydroxide

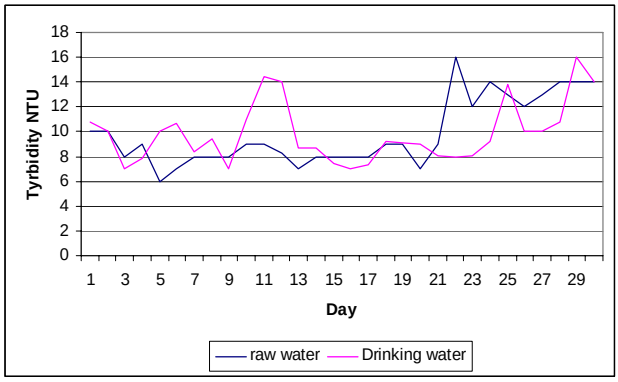

Figure.2 The turbidity of the raw water and obtained drinking water per days

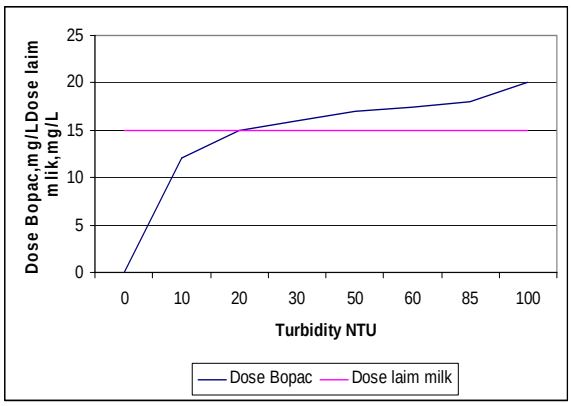

Figure 3. The optimum dose of aluminium base poly chlorine and calcium hydroxid

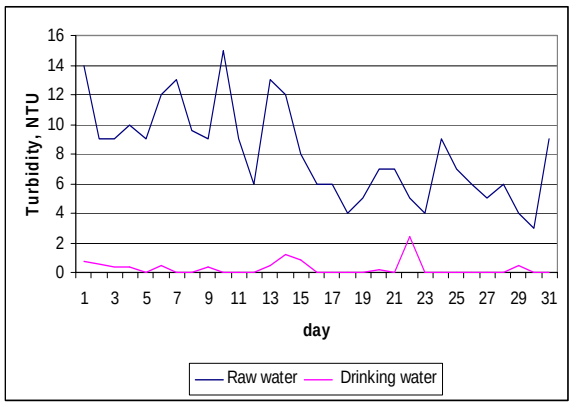

Figure 4. The turbidity of the raw water and obtained drinking water per days

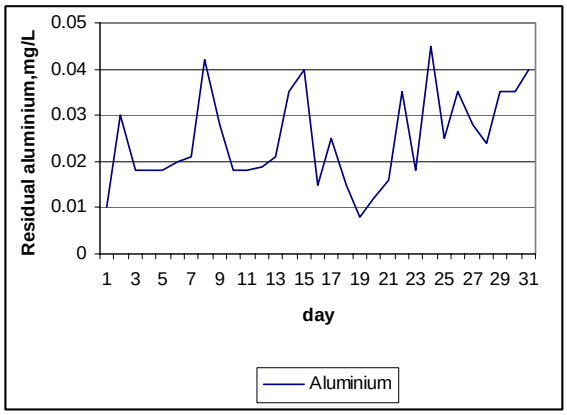

Figure 5. The residual aluminium dependence versus days

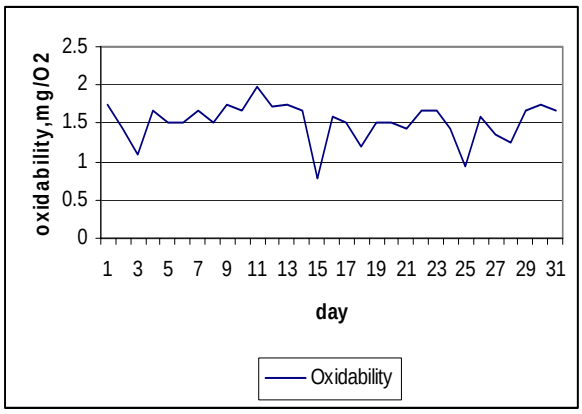

Figure 6. Oxidability dependence versus days versus days

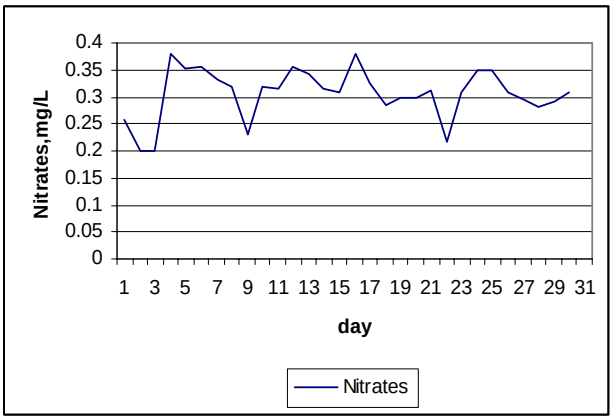

Figure 7. Nitrates dependence versus days

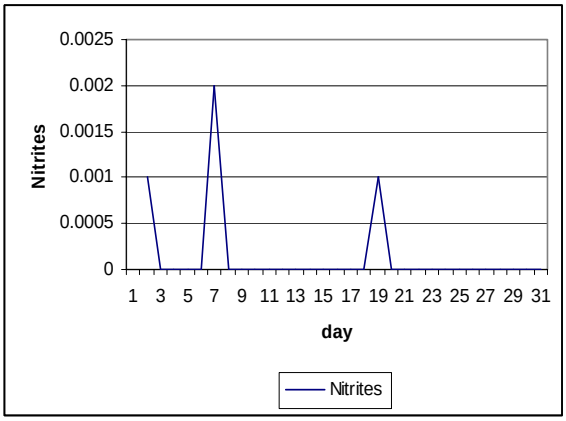

Figure 8. Nitrites dependence versus days

From the experimental data can be observed that all the studied parameters are under the maximum concentration admitted by the water quality legislation (aluminium-CMA = 0.2 mg/L, oxidability-CMA = 5 mg/LO_2, nitrates-CMA = 50 mg/L, nitrites-CMA = 0.5 mg/L).

From the experimental data obtained using the studied treatment technique and applied at the station "Micro plant" allowed the establishment with the help of the Table Curve 2D programme, version 5.01, of the mathematic model equation in the view of the aluminium optimum dose determination versus of the raw water turbidity.

The mathematic model equation is:

$$Y = \frac{a + cX}{1 + bx + dx^2} \qquad (1)$$

where:

a, b, c, d are the coefficients.

a = -0.22887454

b = 0.087370296

c = 1.8861458

d = $-8.51725e^{-0.5}$

4. Conclusions

In the frame of the presented paper was studied comparative two treatment techniques of water applied at the station "Micro plant", treatment technique with aluminium sulphate and calcium hydroxide and aluminium base poly chlorine and calcium hydroxide. Enhanced techniques were studied during one month. The water treatment technique with aluminium sulphate and calcium hydroxide does not present efficiency in the water treatment process because the insufficient time for the proceeding of the coagulation optimum conditions. The turbidity of the obtained treated water in the studied technological flux are not in accordance with the legislation requirements, in conclusion the obtained water can not be drinkable.

The water treatment technique with aluminium base poly chlorine and calcium hydroxide is optimal and present efficiency on the coagulation process of the suspension from water. The obtained experimental values for the treated water for the turbidity, oxidability, residual aluminium, nitrates and nitrites are in accordance with the legislation requirements.

Aluminium bases poly chlorines used in the water treatment present the advantage that react very quickly with the water, assure a good removal of the organic maters from water only by coagulation, determine a small value of residual aluminium in the treated water and correspond to the European requirements. On installation can be dosed also diluted.

The water treatment technique present the advantage that can be applied practical on a small automated installation, this being the major reason of this effectuated study.

The original contribution of the made study allowed the applying in practice of the treatment optimum technique at the station "Micro plant" managed by the SC Vital SA Baia Mare. The obtained drinking water is discharged in present to the inhabitants from the Ferneziu district of the city and is always in accordance with the legislation requirements.

5. References

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UNCERTAINTIES ESTABLISHMENT IN DETERMINING AFLATOXIN M1 ANALYSIS USING ENZYME IMMUNOASSAY TESTS (ELISA)

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Abstract

As a result of ISO 17024 requirements regarding the accreditation of laboratories, an assessment of the uncertainty of the measurements is necessary, having to be expressed in a way that can be transmitted to the customer at request. The present paper describes the method of estimating in the case of the determining of M1 aflatoxin from milk. The uncertainty in measurement for the determining of the M1 aflatoxin through the ELISA method has been calculated starting from the Type A assessment of the standard uncertainty through the statistical analysis and through the Type B assessment of the standard uncertainty as a method of assessing of the uncertainty through other means than the statistical analysis of a series of observations and through the quantification of the two types of uncertainties the combined uncertainty and extended uncertainty have been obtained.

Keywords: *Aflatoxin, uncertainty, calibrators, precision, trueness, ELISA*

1. Introduction

The estimation of the uncertainty is a fundamental part of the ISO 17025 standard for laboratories of trials and calibration, which provides a quantitative assessment of the analytical results.

According to this standard, the laboratories have to have procedures that have to be implemented when any aspect of the works tried and/or calibrated or the work results are not corresponding to their own procedures or with the requirements of the client.

Calibration laboratories or the trial laboratories that make their own calibrations have to have and to apply estimating procedures of the uncertainties in measurement for all the calibrations and types of calibration [1].

This paper describes the estimating process of the uncertainty through the method of determining the aflatoxin in milk ELISA.

This is describes by *Daniels J. M. et. All {2]*as a metabolic hydroxyl of the B1 aflatoxin, which can be encountered in milk or dairy products obtained from animals that have ingested contaminated forage, which is obtained from the B1 aflatoxin as a result of the mono-oxygenase microsomial that transforms the B1 aflatoxin in M1 aflatoxin at the level of the hepatocyte cells.

Therefore at an international and national these issues led to a more strict imposition of new legislative limits concerning some mycotoxins that can contaminate food and raw materials in the food chain.

According to the European Union regulations, the maximum level of aflatoxin M_1 in raw milk, heat-treated milk and milk-based products should not exceed [3, 4].

2. Materials and methods

Milk samples were analyzed using the ELISA method (Enzyme-linked immunosorbent assay) and interpreted according to the instructions of the standard or of the analysis kit (R-Biopharm AG, Ridascreen for AFM_1, Art Nr. R1111).

39 determinations were made using this ELISA kit, samples and standards were analyzed in duplicate.

The concentration of M1 Aflatoxin in milk samples was calculated using the following equation:

$$\%Ab = \frac{A}{A_0}100 \qquad (1)$$

where:

A – absorbance standard, %

A_0 – Absorbance zero standard, %

The zero standard is thus made equal to 100 % and the absorbance values are quoted in percentages. The values calculated for the standards are entered in a system of coordinates on semi logarithmic graph paper against the aflatoxin M1 concentration [ng/l].

In order to obtain the aflatoxin M1 concentration in ng/l actually contained in a sample, the concentration read from the calibration curve must be further multiplied by the corresponding dilution factor.

All the procedures for the ELISA analysis are shown in Figure 1.

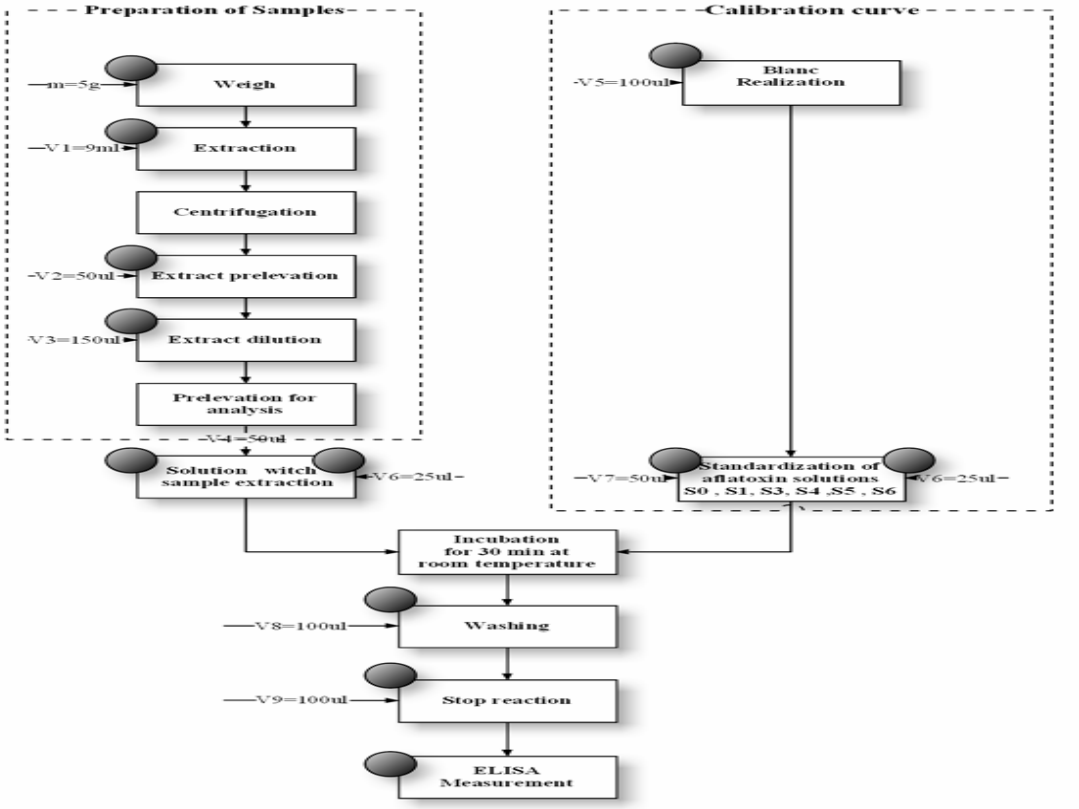

Figure. 1. Procedures for the ELISA analysis
m- sampling quantity ; V_1 – the methanol volume used for extraction ; V_2- the supernatant volume obtained through centrifugation; V_3- the volume of the tampon solution for dilution ; V_4 –the volume of solution used in the analysis ; V_5 – the volume of tampon solution for blank ;V_6 –the volume of solution conjugated ; V_7-the volume of tampon solution for standardizing ; V_8 – the volume of substrata solution ; V_9 – the volume of H_2SO_4 solution for stopping the reaction ; ● -uncertain

3. Results and discussion

3.1. Assessment of Type A of the standard uncertainty

The assessment of Type A of the standard uncertainty can apply when more independent observations have been done of one of the entrance quantities in the same conditions of measurement. If the measurement process has enough resolution, a spreading and scattering of the obtained values will exist [5].

The results of the measurements for the M1 aflatoxin are represented in Table 1.

Table 1. *Experimental data obtained*

No.	Conc. (ppb.)	No.	Conc. (ppb.)
1	0.012	16	0.011
2	0.013	17	0.012
3	0.011	18	0.012
4	0.012	19	0.011
5	0.011	20	0.011
6	0.010	21	0.012
7	0.011	22	0.012
8	0.012	23	0.009
9	0.011	24	0.010
10	0.012	25	0.011
11	0.013	26	0.010
12	0.012	27	0.011
13	0.011	28	0.009
14	0.012	29	0.011
15	0.013	30	0.012

Average:

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i = 0.011333 \qquad (2)$$

Standard experimental deflection:

$$s = \sqrt{\sum_{i=1}^{n} \frac{(x_i - \bar{x})}{n-1}} = 0.00101105 \qquad (3)$$

Relative uncertainty:

$$u_{rA} = \frac{S}{x_i} = 0.08921 \qquad (4)$$

$$u_{rA}^2 = 0.007958 \qquad (5)$$

3.2.Assessment of Type B of the standard uncertainty

The assessment of Type B of the standard uncertainty is the assessment of the associated uncertainty with an estimation of an entrance quantity through other means than statistical analysis.

The assessment of Type B of the standard uncertainty provides as much confidence as an assessment of Type A of the standard uncertainty, especially in the case of a measurement in which the assessment of Type A is based only on

comparatively small number independent statistic observations *(EA-4/02)*

The compound uncertainty has been calculated from the report between the extended uncertainty U and the covering factor which at a probable coverage of 95% is in most of the cases 2

$$u_{cA} = \frac{U}{k} \quad (6)$$

and the relative uncertainty has been obtained in the proportion between the compound uncertainty

$$u_{rA} = \frac{u_c}{\bar{x}} \quad (7)$$

Table 2 presents the data of the uncertainties of Type B for the measurement instruments, in all the cases the covering factor k is 2 for a covering probability of 95%.

Table 2. *Uncertainty data of Type B*

Size	Operation	Instrument of measuring	Extended uncertainty U	Compound uncertainty u_{cB}	Relative uncertainty u_{rB}
M	measuring	XT120A scale	0.2 mg	0.1	0.02
V1	Extraction	Measuring pipette class AS - 10 ml	0.05 ml	0.025	0.0027
V2	Extract assay	Micropipette 50 µl	0.1	0.05	0.001
V3	Dilution extract	Micropipette 150 µl	0.5	0.25	0.0003
V4	Assay for analysis	Micropipette 50 µl	0.1	0.05	0.001
V5	Blanc preparation	Micropipette 100 µl	0.5	0,.25	0.0025
V6	Adding conjugate	Micropipette 25 µl	0.1	0.05	0.002
V7	Preparing etalon	Micropipette 50 µl	0.1	0.05	0.001
V8	Washing	Micropipette 100 µl	0.5	0.25	0.0025
V9	Stopping the reaction	Micropipette 100 µl	0.5	0.25	0.0025
A	Reading at ELISA apparatus	ELISA SINNOWA	0.001	0.005	$8.3x10^{-7}$

$$u_{2Br} = \sum_{i=1}^{11} u_{Bri}^2 = uBrM^2 + uBrV1^2 + uBrV2^2 + uBrV3^2 + uBrV4^2 + uBrV5^2 + uBrV6^2 + UBrV7^2 + uBrV8^2 + uBrV9^2 + uBrA^2 = 0.0355 \quad (8)$$

$$|y| = \bar{x} = 0.011333 \quad (9)$$

$$u_{rA}^2 = 0.08921 \quad (10)$$

$$u_{rB}^2 = 0.0355 \quad (11)$$

Combined uncertainty:

$$\left[\frac{u_c(y)}{|y|}\right] = u_{rA}^2 + u_{rB}^2 \quad (12)$$

$$u_c(y) = |y|\sqrt{u_{rA}^2 + u_{rB}^2} \quad (13)$$

$$u_c(y) = 0.90234\% \quad (14)$$

The extended uncertainty for k = 2, P = 95% is:

$$U = k \cdot u_c(y) = 2 \cdot 0.90234 = 1.80468\% \quad (15)$$

And result is:

$$Y = \bar{x} \pm U = 0.011333\text{ppb aflatoxin} \pm 1.80468\% \quad (16)$$

4.Conclusions

Sensitivity and reproducibility of determinations on aflatoxin M1 are current challenges in developing new analysis methods. These include, mainly, the insurance that the tested sample is representative for a larger lot and that determinations are sensible and precise enough for using them at a larger scale. The analysis conditions of aflatoxins are of such nature that, for most aflatoxins known, the current analysis techniques have overreached these obstacles

The experimental data obtained in this research gives valuable information regarding some features of the specific parameters according to the requirements of SR EN ISO / CEI 17025:2005 and OIE Manual. Also, the whole kit accomplishes the validation parameters, which recommended for use in analysis of aflatoxin M1 in milk.

However ELISA tests are available as an alternative to the chromatographic processes which take time for the routine analysis. They are also available at a large scale, generally being used for screening.

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DEVELOPMENT OF PROBIOTICS ICE CREAM IN PAKISTAN FROM BUFFALO MILK BY USING *B. BIFIDUM* AND L. ACIDOPHILUS

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ABSTRACT

The main objective of this research work was to develop probiotics ice cream containing different concentrations of L. acidophilus and B. bifedum from buffalo milk. Probiotics (L. acidophilus, B. fedum) were added either alone or in combinations in ice cream mix (cfu/mL) at 9 different concentrations i.e. T_1 (Lactobacillus acidophilus 10^6) T_2 (Lactobacillus acidophilus10^7) T_3 (Lactobacillus acidophilus10^8) T_4 (Bifidobacterium bifidum 10^6) T_5 (Bifidobacterium bifidum 10^7) T_6 (Bifidobacterium bifidum 10^8) T_7 (L. acidophilus+ B. bifidum 10^6+10^6) T_8 (L. acidophilus+ B. bifidum 10^7+10^7) T_9 (L. acidophilus+ B. bifidum 10^9+10^9). All these treatments were compared with a control (T_0) which did not contain any addition probiotics culture. The composition of basic formulation (control) comprised 8% milk fat, 10% MSNF, 10% sucrose, 4% liquid glucose, 0.25% sodium alginate and 0.25% glycerol monostearate. The ice cream mixes were fermented at 41 ^{0}C for 8 hours after the addition of probiotics. The addition of both probiotics at all levels did not have any adverse effect on fat and protein content, overrun and melting resistance. pH and acidity of T_3, T_6 and T_9 was 6.38,0.27%; 6.51,0.24% and 5.39,0.39% respectively as compared to control 6.7,0.16%. Buffalo milk based pobiotics ice cream samples were stored at -18 ^{0}C for 90 days to determine the viability during storage. The survival of probiotics bacteria L.acidophilus, B. fedum alone and in combination of L. acidophilus and B. fedum was 65, 88 and 73% respectively. T_3 showed highest percentage of survival rate (89.09%) followed by T_5 (88.20%) and T_4 (80.75%). Lowest percentage of survival rate was found in treatment T_1 (57.20) followed by T_7 (62.85%) and T_2 (66.08). B. bifidum showed better survival rate as compared to L. acidophilus and mixed cultures of L. Acidophillus and B. bifidum. The overall acceptability score of all the treatments were not significantly influenced from each other. The lowest overall acceptability score obtained by T_9 was 6.4 out of 9.

Keywords: *L. acidophilus; B. bifidum*; overall acceptability.

1.Introduction

Ice cream is a frozen dessert made from milk, cream, sugar, and stabilizers, emulsifiers combined with or without flavors, fruits, nuts or other ingredients. Cultured dairy products, in particular, are becoming increasingly popular among the consumers who are quite in tune with intestinal health and dietary means for enhancing well being. Among dairy products with live cultures, probiotic ice-creams or fermented frozen desserts are also gaining popularity [1] . Probiotic food is defined as a food product that contains viable probiotic microorganisms in sufficient populations incorporated in a suitable matrix [2, 3]. Probiotics are basically gut friendly bacteria, which can be consumed directly or can be incorporated in various food items. Different types of products have been proposed as carrier foods for probiotic micro-organisms by which consumers can take in large amounts of probiotic cells for therapeutic effect [4]. It is estimated that more than 70 products containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum,* including sour cream, buttermilk, yoghurt, milk powder and frozen dessert, are produced worldwide [5]. Consumption of probiotic bacteria with ice cream could be an ideal way to re-establish intestinal microflora balance. Therefore, it is important

to ensure a high survival rate of these bacteria during the product shelf life to maintain consumer confidence in probiotic products [6]. The ice-cream matrix might be a good vehicle for probiotic cultures, due to its composition, which includes milk proteins, fat and lactose, as well as other components.

Currently, in Pakistan, the only cultured dairy product which is popular and easily available in the market is yoghurt. The drawback with yoghurt is its short shelf life and also the bacterial culture present in yoghurt as starter culture, is less resistant to the acidic environment of stomach and bile juices. In this regard, the performance of probiotics as fermenting agent as well as to improve the health is more effective than the starter culture of yoghurt. The species of bacteria most commonly used in dairy products for probiotic effect are *Lactobacillus* and *Bifidobacterium* [3]. Buffalo is the major dairy animal in Pakistan and the estimated buffalo population in the country is 29 million heads. Pakistan stands second among buffalo-milk producing countries in the world. Pakistan is fortunate enough in having two best sub-tropical breeds of buffaloes such as Nili-Ravi and Kundi. The best buffalo animals are found in the canal fed areas of the country, where abundant fodder supply and crops by products are available. In Pakistan buffalo milk (19.7 Million tons) is the major dairy animal contributing maximum in total milk production followed by cattle (9.0 Million tons) [7]. Indeed Pakistani buffaloes are the best milch buffaloes in the world. In Pakistan, there is little work has been done regarding probiotic addition in dairy products and no work particularly in buffalo milk based ice cream. Furthermore, no health promoting ice cream product already exists in market**.** Keeping in view importance of probiotic foods, importance of buffalo milk the present study was carried out to develop probiotics ice-cream in Pakistan by using various levels of (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*) and evaluate the suitability of buffalo milk based probiotics ice cream on the basis of certain physico-chemical, microbiological and sensory properties.

2. Materials and methods

2.1. Raw Materials

Buffalo milk was obtained from Buffalo Research Institute, Pattoki, instant skim milk powder, stabilizer, emulsifier, sunset yellow color, vanilla flavor and freeze dried cultures of *Lactobacilus acidophilus* (DVS LA-5 Probio-Tec®) and *Bifidobacterium bifidum* (DVS BB-12® Probio-Tec®) were obtained from Chr. Hansen (Hørsholm Denmark). The cultures were stored at -18 ^{0}C until they are used.

2.2. Cell count of probiotic culture

Initial cell count of both freeze dried probiotic bacteria was determined by pour plate method. One gm of bacterial culture was dissolved in 100 ml autoclaved peptone saline, then 10-fold dilutions were made in autoclaved peptone saline, then 1 ml of each appropriate culture dilution was added to a pre autoclaved Macortny bottle having 20 ml autoclaved MRS agar media, it was shaked well then poured in a sterile Petri dish tempered (approx. 47ºC) and incubated anaerobically for 3 days at 37ºC.

2.3. Experimental Design

L. acidophilus and *B. bifidum* was added at nine different levels i.e. T_1 (*L. acidophilus* 10^6 cfu/mL) T_2 (*L. acidophilus*10^7 cfu/mL) T_3 (*L. acidophilus*10^8 cfu/mL) T_4 (*B. bifidum* 10^6 cfu/mL) T_5 (*B. bifidum* 10^7 cfu/mL) T_6 (*B. bifidum* 10^8 cfu/ mL) T_7 (*L. acidophilus*+ *B. bifidum* 10^6+10^6 cfu/ mL) T_8 (*L. acidophilus*+ *B. bifidum* 10^7+10^7 cfu/ mL) T_9 (*L. acidophilus*+ *B. bifidum* 10^9+10^9 cfu/

mL). All these treatments were compared with a control T_0 which did not contain any probiotic culture. Each treatment was replicated 3 times. The mix was heated to 80°C, homogenized in a clean and sanitized double stage homogenizer (APV) at 100 kg/cm^2 and 35 kg/cm^2 pressure in the first and second stage respectively. Probiotic Ice cream was manufactured in the pilot plant of the Research and Development Department, Unilever Walls Ice Cream Factory Lahore, Pakistan. One gm freeze dried culture for *L. acidophilus* contained = 8.4 x 10^9 cfu and one gm freeze dried culture for *B.bifidum* contained = 7.5 x 10^9 cfu. From these guidelines the quantities of probiotics cultures were calculated as 1000 mg= 10^9, 100 mg= 10^8, 10 mg= 107 and 1mg= 10^6. The composition of basic formulation (control) comprised 8% milk fat, 10% MSNF, 10% sucrose, 4% liquid glucose, 0.25% sodium alginate and 0.25% glycerol monostearate. The experiments involved making of 10 types of ice cream i.e., T_1 to T_9 along with control T_0.

2.4. Inoculation of probiotic culture in ice cream mix

Ice cream mix was divided into ten equal parts. Each part was inoculated by probiotic culture according to the experimental design except for control. Each sample was mixed well and kept for fermentation for two hours at 41°C. Mix was cooled at 4°C; vanilla flavor was added in it and aged for 24 hours followed by freezing and hardening. It was packed manually in 1 liter plastic tubs and was first frozen at -5°C at -18 °C.

2.5. Analysis

Ice cream samples were stored at -18 °C ± 2°C and were analyzed for physical, chemical, microbiological and sensory evaluation. Melting resistance, overrun and viscosity was determined by using the procedures as described by Upadhyay *et al*. [8]. pH, acidiy, fat, protein, total solids content was determined following the respective procedures as prescribed in AOAC [9]. Reinforced Clostridial Agar (RCA) (Oxoid) was used as a media to enumerate *L. acidophilus* and *B. bfidum* (Ming, 1989) in probiotic ice cream samples. RCA media was prepared by following the instructions of manufacturer (Oxoid). Frozen fermented ice cream was thawed and then diluted in autoclaved 0.85% saline. With the help of micropipette ,one-tenth milliliter of each dilution was placed on the centre of solidify RCA plates and spread evenly over the surface by means of a sterilized bent-glass-rod The plates were then incubated in an anaerobic environment (Anaerogas Pack Himedia ref LE 002A-5NO) at 37ºC for 48 hours. The total numbers of *L. acidophilus* and *B. bifidum* were determined based on their colony morphology when grown on RCA. *L. acidophilus* produced pinpoint-sized colonies, but B. *bifidum* produced large colonies.

2.6. Sensory evaluation

Ice cream samples prepared from all the treatments of probiotic cultures together with regular (control) were subjected to sensory evaluation by trained taste panel of five judges. Evaluation was done by using nine (9) point hedonic scale on a sensory evaluation Performa for following parameters; color, thickness, flavor, taste and overall acceptability. All the samples were coded with three digit random number and all orders of serving were completely randomized [10].

2.7. Statistical analysis

The data was obtained by using Completely Randomized Design (CRD) and outcome of the analysis was analyzed through two way analysis of variance under factorial arrangement to find out the effect of treatment and effect of storage time on probiotics ice cream by following the

method of [11]. Significance in means was compared using Duncan's Multiple Range test [12].

3. Results and discussion

As can be seen from the results of Table 1, the addition of probiotics at T3 level (*L. acidophilus108* cfu/50 mL) and T9 (*L. acidophilus*+ *B. bifidum* 109+109 cfu/50 mL) imparted as significant lowering effect on pH of ice cream. The treatments containing *L. acidophillus* alone or in combination with *B. bifedum* showed lower pH as compared to treatments augmented with bifedo bacteria only and T_0 (without any augmentation of probiotics). As the concentration of *L.acidophillus* was increased in the treatments alone or in combination with bifedo bacteria more acid was produced. Addition of bifedo bacteria alone at T_4 (*B. bifidum* 106 cfu/50 mL) and T5 (*B.* 107 cfu/50 mL) level did not have significant adverse affect on pH and acidity, addition of *B. bifedum* at T6 level (*B. bifidum* 108 cfu/50 mL) significantly lowered the pH (6.51) as compared to control (6.7) of ice cream. Significanty the lowest pH was recorded in samples treated with *L. acidophilus,* comparatively high pH was recorded in samples inoculated with *B. bifidum. B. bifedum* is not a good acid producer while *L. acidophillus* is a high acid producer which may be the probable reason for low pH and high acidity in treatments containing these bacteria. Turgut and Cakmakci, [13] studied the effect of different probiotic cultures in ice cream mixes and reported that ice cream mixes containing *L.acidophilus* had significantly ($P<0.05$) higher acidity and lower pH as compared to those samples containing *B. bifedum*. Akalin [14] while studying the effect of effects of inulin and sugar levels on the viability probiotic, physical and sensory characteristics of probiotic ice-cream reported that all the experimental samples had nearly the same composition but more acid was produced in samples containing *L. acidophilus*. The effect of different concentrations of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* did not have any significant effect on overrun ($P>0.05$) of all the treatments along with control. The highest overrun was observed in control and non significantly the lowest overrun 78.60% was found in T9 (*L. acidophilus*+ *B. bifidum* 10^9+10^9 cfu/50 mL), this non significant decline in overrun might be due to the production of more acid during fermentation of ice cream mix. Fat, solids not fat, stabilizers and emulsifiers contribute in overrun of ice cream; the concentrations of these ingredients were same in the formulation of all the treatments which may be reason for almost uniform overrun in all the experimental samples. Akalin and Erisir [14] did not observed any difference in overrun of ice cream prepared with the addition of strains i.e., *L. acidophilus* La-5 or *B.animalis* Bb-12. The average values of compositional attributes of the experimental ice cream samples are product values having 8% fat and 36.10 % total solids. The data showed that there were no discernable differences ($P>0.05$) which are given in Table 1. All the mean percentages of fat, total solids and protein content were close to the control ($P>0.05$) in the composition of the control and experimental treatments. The insignificant variation in the compositional attributes of different types of mix used was probably due to the non variations in the composition of the raw materials.

3.1. Enumeration of Probiotics

Bacteria probiotic ice cream (Table 1) showed that melting resistance was significantly ($P<0.05$) affected by treatments. Highest melting resistance was offered by treatment T_0 (control) whereas least resistance was offered by T3. Ice cream samples inoculated with *B. bifidum* showed better melting

Table 1. *Effect of Treatment on composition of probiotics ice cream*

Treatments	pH	Acidity (%)	Fat (%)	Protein	* TS (%)	*MR Minutes	Overrun (%)
To	6.70±0.54a	0.16±0.05	d	8.86±0.87a	4.05±0.54a	36.02±2.34a	3.54±0.45a
81.03±4.34a							
T_1	6.68± 0.52a	0.16±0.04d		8.81±0.84a	4.11±0.51a	35.85±2.13a	3.53±0.39a
80.87±3.99a							
T_2	6.65± 0.51a	0.19±0.07c		8.84±0.65a	4.06±0.46a	35.93±1.89a	3.12±0.44c
80.62±4.23							
T_3	6.38± 0.47c	0.27±0.09	b	8.86±0.90a	4.02±0.65a	35.94±1.99a	3.53±0.34a
79.81±3.85a							
T_4	6.69± 0.52a	0.16±0.04d		8.87±1.12a	4.09±0.48a	36.03±2.04a	3.54±0.56a
80.83±4.67a							
T_5	6.68± 0.45a	0.17±0.05	d	8.88±0.99a	4.05±0.38a	36.08±2.11a	3.22±0.29b
80.59±4.94a							
T_6	6.51± 0.42b	0.24±0.08	b	8.87±0.97a	4.10±0.44a	35.93±1.84a	3.55±0.37a
79.90±3.57a							
T_7	6.66± 0.46a	0.19±0.05c		8.82±1.42a	4.03±0.57a	36.03±2.45a	3.26±0.67b
80.63±4.56a							
T_8	6.53± 0.38b	0.21±0.07	c	8.88±1.23a	4.01±0.68a	36.02±1.56a	3.19±0.41c
80.20±4.78a							
T_9	5.89± 0.27d	0.39±0.12	a	8.84±0.93a	4.07±0.36a	36.06±1.68a	3.36±0.54b
81.03±4.11a							

Means of triplicate experiments; means with same letters in columns are statistically non significant by Tuckey's T-Test at 0.05 level of significance.
*TS- Total Solids
MR- Melting Resistance
To(control without probiotics)
T_1(*Lactobacillus acidophilus*10^6 cfu/50 mL)
T_2(*Lactobacillus acidophilus*10^7 cfu/50 mL)
T_3(*Lactobacillus acidophilus*10^8 cfu/50 mL)

T_4(*Bifidobacterium bifidum* 10^6 cfu/50 mL)
T_5(*Bifidobacterium bifidum* 10^7 cfu/50 mL)
T_6(*Bifidobacterium bifidum* 10^8 cfu/50 mL)
T_7(*L. acidophilus*+ *B. bifidum* 10^6+10^6 cfu/50 mL)
T_8(*L. acidophilus*+ *B. bifidum* 10^7+10^7 cfu/50 mL)
T_9(*L. acidophilus*+ *B. bifidum* 10^9+10^9 cfu/50 mL)

Table 2. *Viability count for probiotic bacteria log (cfu/ml)*

Treatments	O-Day	15-Days	30-Days	45-Days	60-Days	75-Days	90-Days	*SR(%)
T1	5.87± 0.67d	5.59±0. 54d	5.82±0. 44^e	5.92±0. 38^e	3.73±0. 28f	3.43±0. 34f	3.36±0. 35f	57.20
T2	8.52± 0.43b	8.86±0. 97b	7.55±0. 69c	6.75±0. 46d	5.89±0. 47d	5.73±0. 54d	5.63±0. 45d	66.08
T3	9.12± 0.75a	9.75±0. 99a	8.65±0. 73b	8.83±0. 87b	7.46±0. 69b	6.77±0. 68c	6.54±0. 57c	71.74
T4	6.96± 0.61c	6.81±0. 65c	6.56±0. 57d	5.57±0. 56^e	5.75±0. 55d	5.69±0. 77a	5.62±0. 47d	80.75
T5	8.93± 0.85b	8.58±0. 76b	8.76±0. 78b	8.83±0. 77b	7.66±0. 87b	7.740±0 .78b	7.88±0. 98b	88.20
T6	9.80± 0.99a	9.81±0. 97a	9.75±0. 88a	9.56±0. 96a	8.66±0. 89a	8.85±0. 69a	8.73±1. 04a	89.09
T7	6.86± 0.75c	6.76±0. 59c	5.35±0. 42^e	5.46±0. 44^e	4.39±0. 46e	4.32±0. 48^e	4.32±0. 68^e	62.85
T8	8.74± 0.81b	8.59±0. 79b	8.48±0. 73b	7.99±0. 65c	6.75±0. 58c	6.69±0. 87c	6.65±0. 77c	76.11
T9	9.59± 1.23a	9.44±0. 95a	9.35±0. 89a	8.84±0. 88b	8.82±0. 92a	7.72±0. 89b	7.71±0. 82b	80.36

*Survival Rate
Means of triplicate experiments; means with same letters in rows and columns are statistically non significant by Tuckey's T-Test at 0.05 level of significance.

To(control without probiotics)
T_1(*Lactobacillus acidophilus*10^6 cfu/50 mL)
T_2(*Lactobacillus acidophilus*10^7 cfu/50 mL)
T_3(*Lactobacillus acidophilus*10^8 cfu/50 mL)
T_4(*Bifidobacterium bifidum* 10^6 cfu/50 mL)
T_5(*Bifidobacterium bifidum* 10^7 cfu/50 mL)
T_6(*Bifidobacterium bifidum* 10^8 cfu/50 mL)
T_7(*L. acidophilus*+ *B. bifidum* 10^6+10^6 cfu/50 mL)
T_8(*L. acidophilus*+ *B. bifidum* 10^7+10^7 cfu/50 mL)
T_9(*L. acidophilus*+ *B. bifidum* 10^9+10^9 cfu/50 mL)

resistance as compared to *L. acidophilus.* These results might be due to less acid production property of *B. bifidum*. The melting resistance of ice cream was decreased by increasing concentration of bacteria. Kebary [15] Studied the viability of *B. bacterium bifidum* and its effect on quality of frozen Zabady (frozen dessert) and reported that all the samples had nearly the same melting resistance.

L. acidophilus and *B. bifidum* both were able to grow and produce acid during fermentation in ice cream mix (Hekmat and McMahon [16]. At 0 day (-18 ºC), 0 day the total number of colonies counted were 10^5 cfu/ml in ice cream samples inoculated with treatment T1, T4 and 10^6 in case of T7. Treatments T2, T5 and T8 showed comparatively better colonies i.e., 10^8 cfu/ml. T3, T6 and T9 showed maximum number of bacteria i.e., 10^9 cfu/ml. The probiotic culture added in combination i.e. treatments T7, T8 and T9 showed results close to treatments having single culture. The reason might be nutrients competency for both bacteria. Nutrients requirements for *B. bifidum* is more than *L. acidophilus* [17]. Storage at -18ºC showed gradual decrease in number of bacteria. First reduction was observed after 45 days. There was total reduction of approximately 3 log cycles in case of *L. acidophilus* whereas, viability loss was observed only 1 log cycle for *Bifidobacterium bifidum* in the total experimental duration of ninety days. Hekmat and McMahon [16] observed in their studies that the viability of *L. acidophilus* in a standard ice cream mix decreased by 2 log cycles after storage for 17 weeks at -29 ºC. In addition to that, more viability loss was observed in case of *L. acidophilus* which may be due to its more acid production property than *Bifidum*. The decline in viable bacterial count during freezing step is most likely to be due to actual freezing of the cells that caused death of some cells. However, the mechanical stresses of the mixing and freezing processes and also oxygen incorporation into mix may have resulted reduction in viable bacterial count. Similar results were reported by earlier studies [18, 19, 20]. It was also observed that after 15 and 45 days of storage no decrease was found in viable count. However, a decrease in viable count was observed during 45 to 90 days of storage at -18 ºC, present study can be concluded as ice cream can serve as a good mean for delivering probiotic bacteria to the consumer. *B. bifidum* and *L. acidophilus* exhibited approximately similar properties. Level of probiotic bacteria used in treatment T3, T5 and T6 was found more appropriate in making ice cream a probiotic food product. *B. bifidum* was found better in maintaining viable count i.e., 10^6 to 10^7 cfu/ml at the time of consumption after three months. Its survival rate was maximum among all treatments (89.09%).

3.2.Effect on sensory attributes of ice cream

The results depicted in Table 3 represent the organoleptic attributes of ice cream as adjudged by a panel of five judges in terms of color, taste, surface spots, thickness and overall acceptability. It is evident from statistical analysis of score obtained, that various treatments did not impart any significant difference on color of probiotic ice cream. Color plays an important role in making a food product appealing and eatable for the consumer. Parameter of surface spot was studied to check any impression, secretion, mark or spot produced by probiotic bacteria added in ice cream. No sign for colony growth and spots of any kind was found on surface of ice cream which indicated more acceptance of the product. It means both bacteria have excellent capability of addition in ice cream. Increased levels of bacteria had a negative effect on thickness of ice cream. The low scores of thickness of T_1, T_2, T_3, T_6, T_7, T_8 and T_9 and may be attributed to the more

acid producing capacities of *L. acidophilus* and more number of bacteria in these treatments. Probiotics bacteria are acid producing in nature, so they gave a mild sour taste to ice cream. Strong influence was found in samples inoculated with treatment T_3. Change in taste was correlated with added number of bacteria**.** Overall is a combination of all other sensory parameters like colour, flavour, thickness, taste etc. Although there were slight variations observed in some of the sensory parameters as compared to the control. Addition of probiotic bacteria in ice cream had a slight negative effect on overall acceptability in T_3 and T_9. The decline in acceptability scores may be attributed to the higher numbers of bacteria alone or in combination. So it can be concluded from the scores of acceptability conclusively from the results that ice cream can act as good source for delivering probiotic to consumer without bringing much change in the nature of product.

4. Conclusions

The main objective of this research work was to develop probiotics ice cream from bufflo milk containing different concentration of L. acidophilus and B. fideum in Pakistani environment. The addition of probiotics bacteria on all levels did not have any significant negative effect on compositional attributes. Overrun and melting resistance of ice cream prepared by augmenting different levels of probiotics was not significantly influenced from the control. The survival of probiotics bacteria *L. acidophilus, B. fedum* and in combination *L.acidophilus, B. fedum* in buffalo milk based ice cream was 65, 88 and 73% after storage of 90 days. The lowest overall acceptability score obtained by T_9 was 7 out of 9 which was more than 77%. Hence buffalo milk can be used in the manufacturing of probiotics ice cream containing *L.acidophilus, B. fedum* with acceptable sensory quality.

5. References

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INVESTIGATION OF THE NUTRITIVE VALUE OF NATURAL, TWO POTENTIAL PLANT-PRODUCT FOOD

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ABSTRACT

We report compositional data for two plant foods that comprise the daily diets of south Iran. Nutritive analysis of Jacques root tubers and Spine Gourd fruits were evaluated. The root tubers contained a ashes: 10.38%,crude protein: 4.54%, crude lipid: 2.00%, crude fiber: 17.24%, and carbohydrates: 65.84%. The fruits also have high energy value (299.52kcal/100g)dry weight where as the fruits contained a ashes: 9.1%,crude protein: 5.44%, crude lipid: 3.25%, crude fiber: 22.9%, and carbohydrates: 59.31%. The fruits also have high energy value (288.25kcal/100g)dry weight. Mineral ranges (mg/100g dry weight, DW) were: K (4.63), Na (1.62), Ca (7.37), Fe (5.04), and Zn (3.83). Comparing the root tubers mineral contents with recommended dietary allowances (RDA), the results indicated that Jacques root tubers could be a good supplement for some nutrients such as calcium, fibre and carbohydrates where as the results indicated that Spine Gourd fruit could be a protein, lipid, potassium and Zinc, fibre and carbohydrates. The root tubers and the stem could be promoted as a carbohydrate supplement for cereal-based diets in poor rural communities, while its high potassium content could be utilized for the management of hypertension and other cardiovascular conditions.

Keywords: *Jacques, Spine Gourd,micronutrients, proximate and mineral composition.*

1. **Introduction**

Many wild plant and animal food resources are eaten across Africa in addition to the more commonly known agricultural foods and domesticated animals. We analyzed several of these foods that comprise a significant portion of the annual diet among the Hadza foragers who live in an area surrounding alkaline Lake Eyasi in northern Tanzania [1-3]. The region, which is semiarid with marked wet and dry seasons, encompasses open grassy plains, woodland/ savanna, and wooded riverbeds. Due to inter- and intra-annual variability, there is no general consensus concerning the seasonal availability of specific foods,[4].

In developing nations, numerous types of edible wild plants are exploited as sources of food hence provide an adequate level of nutrition to the inhabitants. Recent studies on agro pastoral societies in Africa indicate that these, plant resources play a significant role in nutrition; food security and income generation [5].

Furthermore, Food and Agricultural Organization (FAO) report, at least one billion people are thought to use wild foods in their diet [6]. In Ghana along, the leaves of over 300 species of wild plants and fruits are consumed. In Swaziland, wild plants provide a greater share of the diet than domesticated cultivars. In India, Malaysia and Thailand, about 150 wild plants species have been identified as sources of emergency food [7]. Similarly, in South Africa about 1400 edible plant species are used, In Sahel region of Africa, over 200 wild foods were identified to be used by the rural communities. In most of these reports, it was emphasized that nutritionally, these unconventional plants foods could be comparable to or even sometimes superior to the introduced cultivars [8]. It is, therefore, worthwhile to note that the incorporation of edible wild and semi- cultivated plant

resources could be beneficial to nutritionally marginal populations or to certain vulnerable groups within populations, especially in developing countries where poverty and climatic changes are causing havoc to the rural populace. In this context, analyses were carried out to evaluate the nutritional content of Jacques root tubers and Spine Gourd fruits with hope that it would be incorporated into the food basket of the country [9]. The present work aimed at determining the chemical composition, nutritive value of Jacques root tubers and Spine Gourd fruits.

2. Materials and Methods

2.1. Plant material

Jacques root tubers and Spine Gourd fruits used as experimental material were collected from farm lands in around Behbahan, South Iran, in October 2007. The collected plant material was placed in a polyethylene bag to prevent loss of moisture during transportation to the laboratory.

2.2. Preparation of the plant material for chemical analyses

Jacques root tubers and Spine Gourd fruits were washed with distilled water and dried at room temperature to remove residual moisture, then placed in paper envelope and oven-dried at 55ºC for 24 hours [10]. The dried root tubers were ground into powder using pestle and mortar, and sieved through 20-mesh sieve. The stem powder was used for the nutrients analyses.

2.3. Proximate analysis

The methods recommended by the Association of Official Analytical Chemists (AOAC) were used to determine ash (#942.05), crude lipid (#920.39), crude fibre (#962.09) and nitrogen content (#984.13)[4].

2.4. Determination of crude lipid and crude fibre content

Two grams of dried samples were weighed in a porous thimble of a Soxhlet apparatus, with its mouthed cotton wool plugged. The thimble was placed in an extraction chamber which was suspended above a pre-weighed receiving flask containing petroleum ether (b.p. 40-60ºC). The flask was heated on a heating mantle for eight hours to extract the crude lipid. After the extraction, the thimble was removed from the Soxhlet apparatus and the solvent distilled off. The flask containing the crude lipid was heated in the oven at 100ºC for 30 minutes to evaporate the solvent, then cooled in a dessicator, and reweighed. The difference in weight was expressed as percentage crude lipid content.

Crude fibre was estimated by acid-base digestion with 1.25% H_2SO_4 (prepared by diluting 7.2 ml of 94% conc. acid of specific gravity 1.835g ml-1 per 1000 ml distilled water) and 1.25% NaOH (12.5 g per 1000 ml distilled water) solutions. The residue after crude lipid extraction was put into a 600 ml beaker and 200 ml of boiling 1.25% H_2SO_4 added. The contents were boiled for 30 minutes, cooled, filtered through a filter paper and the residue washed three times with 50 ml aliquots of boiling water. The washed residue was returned to the original beaker and further digested by boiling in 200 ml of 1.25% NaOH for 30 minutes. The digest was filtered to obtain the residue. This was washed three times with 50 ml aliquots of boiling water and finally with 25 ml ethanol. The washed residue was dried in an oven at 130ºC to constant weight and cooled in a dessicator. The residue was scraped into a pre–weighed porcelain crucible, weighed, ashed at 550ºC for two hours, cooled in a dessicator and reweighed. Crude fibre content was expressed as percentage loss in weight on ignition [11].

2.5. Determination of nitrogen content and estimation of crude protein

Macro–Kjeldahl method was used to determine the nitrogen content of the stem. 2g of dried samples were digested in a 100 ml Kjeldahl digestion flask by boiling with 10 ml of concentrated tetraoxosulphate (VI) acid and a Kjeldahl digestion tablet (a catalyst) until the mixture was clear. The digest was filtered into a 100 ml volumetric flask and the solution made up to 100 ml with distilled water. Ammonia in the digest was steam distilled from 10 ml of the digest to which had been added 20 ml of 45% sodium hydroxide solution. The ammonia liberated was collected in 50 ml of 20% boric acid solution containing a mixed indicator. Ammonia was estimated by titrating with standard 0.01 mol L-1 HCl solution. Blank determination was carried out in a similar manner. Crude protein was estimated by multiplying the value obtained for percentage nitrogen content by a factor of 6.25 [4].

2.6. Estimation of carbohydrates and energy values

Available carbohydrate was estimated by difference, by subtracting the total sum of percent crude protein, crude lipid, crude fibre and ash from 100% DW of the fruit The plant calorific value (in kJ) was estimated by multiplying the percentages of crude protein, crude lipid and carbohydrate by the factors 16.7, 37.7 and 16.7 respectively [12].

2.7. Mineral analysis

The mineral elements Na, K, Ca, Fe, and Zn were determined on 0.3g samples powder by the methods of Funtua (Funtua 2004**;**Funtua and Trace 1999). using Energy Dispersive X-ray Fluorescence (EDXRF) transmission emission spectrometer carrying an annuar 25 mCi 109Cd isotopic excitation source that emits Ag-K X-ray (22.1 keV) and a Mo X-ray tube (50KV, 5mA) with thick foil of pure Mo used as target material for absorption correction. The system had a Canberra Si (Li) detector with a resolution of 170eV at 5.9keV line and was coupled to a computer controlled ADCCard (Trump 8K). Measurements were carried out in duplicate. Na was analyzed after wet digestion of one gramme of the root tubers powder with nitric/perchloric/sulphuric acid (9:2:1 v/v/v) mixture. Sodium was analyzed with a Corning 400 flame photometer [13].

3. Results and Discussion

3.1. Proximate analysis

The results of proximate composition of Jacques root tubers and Spine Gourd fruits are shown in Table 1 and 2. The ash content, which is an index of mineral contents, for Jacques root tubers and Spine Gourd fruits the value of 10.38% DW was less than to the values reported for other edible leaves such as *Momordica balsamina* leaves (18.00 ± 1.27% DW)[14,15]. It is apparent that Jacques root tubers are a good source of calcium where as Spine Gourd fruits are a good source of potassium, and zinc. The root tubers crude protein content (4.54%) was less than where as stem crude protein content (19.38%) was higher than what is reported for some lesser known wild leafy vegetables such as *Momordica balsamina* (11.29 ± 0.07%), *Moringa oleifera* (20.72%), *Lesianthera africana* leaves (13.10 –14.90%) and *Leptadenia hastate* (19.10%)[16,17], plant food that provide more than 12% of their calorific value from protein are a good source of protein. In that context, Jacques root tubers (4.54%) and Spine Gourd fruits (19.38%) are a relatively good source of protein. The crude lipid content (2.00%) of the root tubers and crude lipid content (4.7%) of the stem was less than the range (8.3 – 27.0% DW) reported for some vegetables consumed in Nigeria and Republic of Nigerian [18].

Table 1. *Proximate composition of Jacques root tubers*

Parameters	Concentration, %
Ash	10.38± 0.80
Crude protein	4.54± 0.27
Crude lipid	2.00± 0.50
Crude fibre	17.24± 0.35
Carbohydrates	65.84±0.68
Calorific value(kcal/100g)	299.52±5.31

* The data are mean values ± deviation(SD) of three replicates.
* Values expressed as % wet weight.

Table 2. *Proximate composition of Spine Gourd fruit*

Parameters	Contents, %
Ash	6.7±5.17
Crude protein	19.38± 0.27
Crude lipid	4.7± 0.50
Crude fibre	21.3± 0.35
Carbohydrates	47.92±0.68
Calorific value(kcal/100g)	311.5±5.31

* The data are mean values± deviation(SD) of three replicates.
* Values expressed as % wet weight.

Table 3. *Mineral composition of Jacques root tubers*

Mineral	Recommended Dietary Allowances(mg/day)				
	Available mg/100gDW*	Children	Adult male	Adult female	Pregnant & Lactating Mothers
Potassium	4.29±0.02	800	800	800	1200
Calcium	13.14±0.15	1600	2000	2000	2000
Sodium	3.95±0.08	400	500	400	500
Iran	1.89±0.01	10	10	15	13
Zinc	0.76±0.07	10	15	12	19

* The data are mean values± deviation(SD) of three replicates.

Table 4. *Mineral composition of Spine Gourd fruit*

Mineral	Recommended Dietary Allowances(mg/day)				
	Available mg/100gDW*	Children 7-10 years	Adult male	Adult female	Pregnant & Lactating Mothers
Potassium	0.46±0.02	800	800	800	1200
Calcium	8.25±0.15	1600	2000	2000	2000
Sodium	1.51±0.08	400	500	400	500
Iran	0.14±0.01	10	10	15	13
Zinc	1.34±0.07	10	15	12	19

* The data are mean values± deviation(SD) of three replicates.

The estimated carbohydrate contents (65.84%) in Jacques root tubers and (47.92%) in Spine Gourd fruits was stand to be higher than that for *Senna obtusfolia* leaves (20%) and *Amaranthus incurvatus* leaves (23.7%). On the other hand, *Chlorophytum comocum* root tubers and Spine Gourd fruits contain comparable amount of carbohydrate for *Momordica balsamina* (39.05 ± 2.01%). The crude fibre content in *Chlorophytum comocum* root tubers (17.24 %) in Spine Gourd fruits (21.3 %) was more than the reported values (8.50 – 20.90%) for some Nigeria vegetables [19,21]. One discussed drawback to the use of vegetables in human nutrition is their high fibre content, which may cause intestinal irritation and a decrease of nutrient bioavailability. The fibre RDA values for children, adults, pregnant and breast-feeding mothers are 19 –25%, 21 – 38%, 28% and 29% respectively. Thus, Jacques root tubers and Spine Gourd fruits could be a valuable source of dietary fibre in human nutrition. The calorific value of Jacques root tubers and for Spine Gourd fruits was estimated to be 299.52 kcal/100g (DW) and 311.5 kcal/100g (DW) respectively which is an indication that it could be an important source of dietary calorie. High calorific content of the root tubers could be attributed to high carbohydrates content.

3.2. Mineral content

Table 3 and 4 shows the results of the mineral concentrations of Jacques root tubers and Spine Gourd fruits. Nutritional significant of elements is compared with the standard recommended dietary allowance. When compared with standard values as showed in Table 3, Jacques root tubers less than adequate level of K, Fe, Zn, Ca, and Na, but the plant stem could be a good source of calcium while the plant stem could be good source of K, Na and zinc.

4. Conclusions

The results of the nutritional analysis shown that Jacques root tubers is good sources of plant calcium, carbohydrates where as Spine Gourd is good sources of plant fibre, potassium, Sodium, zinc, lipid and carbohydrates. The results suggests that the plants root tubers and the stem if consumed in sufficient amount could contribute greatly towards meeting human nutritional requirement for normal growth and adequate protection against diseases arising from malnutrition. From the result, Jacques root tubers and Spine Gourd are recommend for continues used for nutritional purposes, considering to the amount and diversity of nutrients it contains. Chemical analysis alone however, should not be the exclusive criteria for judging the nutritional significance of a plant parts.

Thus, it becomes necessary to consider order aspects such as presence antinutritional/ toxicological factors and biological evaluation of nutrient content[20].

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STUDIES ON EFFECT OF DIFFERENT VARIETIES OF DATE PALM PASTE INCORPORATION ON QUALITY CHARACTERISTICS OF YOGHURT

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ABSTRACT

Yoghurt was prepared by using two different varieties of date palm paste cultivars viz. *Behri* and *Safri* at varying levels of concentration. The efforts were made to investigate the influence of different varieties of date paste on physical properties (settling time and synersis), chemical characteristics (pH, acidity, moisture, fat, protein and total soluble solids), sensory quality (appearance, colour, flavour, taste, texture and overall acceptability). The data generated during present investigation revealed that date palm pastes from both varieties could be use as a novel ingredient in enhancing the quality characteristics of yoghurt. Yoghurt sample prepared with incorporation of 10 per cent date palm paste of *Safri* variety resulted in superior organoleptic as well as chemical characteristics compared to other treated samples, justifying its suitability in date palm paste yoghurt preparation.

Keywords: *Phoenix dactylifera*, date palm paste, yoghurt, *Behri*, *Safri*, quality.

1. Introduction

Yoghurt is a cultured milk product and generally fermented with mixture of two species i.e. *Lactobacillus bugaricus* and *Streptococcus thermophilus.* Usually it contains 12-14 per cent total milk solids and has a soft, friable custard like consistency and a clean distinct acid flavour. Yoghurt supplies high quality of protein and is excellent source of calcium, phosphorus and potassium and contains significant quantities of general vitamins [1]. In recent years, yoghurt has become a popular vehicle of incorporating the probiotic species viz. *L. acidophilus* and *B. bifidium* [2]. Yoghurt is valuable adjunct to any healthy diet and the presence of α-D-galactosidase activity in probiotic yoghurt indicates its suitability for lactose-intolerant infants [3].Yoghurt is valued for controlling the growth of bacteria and incurring intestinal diseases like constipation, diarrohea and dysentery. Yoghurt is also effective in curing the blood cholesterol [4]. As milk protein, fat, and lactose components undergo partial hydrolysis during fermentation, yoghurt is an easily digested product of milk [5, 6].

Date palm (*Phoenix dactylifera* L.) is one of the oldest fruit trees in the world [7]. Different Date palm varieties have been developed by thousands of years of selection of seedlings and only those possessing desirable characteristics have been propagated. Most commonly grown date varieties are *Berhi, Dayri, Deglet, Noor, Halawy* ('Halawi'e), *Khadrawy* ('Khadrawi'), *Safri*, *Sayer*.

Date paste used in variety of food preparation by chefs all around worlds, has a rich content of minerals like potassium that are vital for human development while it is free in unnecessary components like sodium and cholesterol. Date paste is largely used as a thickening and gelling agent in different types of processed food products like jams,

jellies, soft cheeses, yoghurts and various confectionaries like cookies, cakes, breads and muffins. It is rich in various nutrients, from appetizers and main dishes to desserts and beverages. Date paste is today added to a large variety of food preparations [8]. The date fruit, because of its tannin content, is used medicinally as a detersive and astringent in intestinal troubles. It is taken to relieve fever, cystitis, gonorrhea, edema, liver and abdominal troubles, and it is also said to counter-react alcohol intoxication [9].

Recently there has been increasing trends to fortify the product with fruits. Generally, plain yoghurt is fortified with fruit to get the fruit yoghurt. Fruits may be used in fresh, freeze, dried or canned state. The amount of fruit added may range from 10 to 30 per cent [10]. Date paste recognized its utility in various value added food products like jam, jelly, beverages and confectioneries. It was hypothesized that date paste could also be utilized as a value enhancer in case of milk based fermented product like yoghurt. Hence, in present investigation, efforts were made to design a research project for preparation of date paste from two different varieties viz. *Behri* and *Safri*, and its utilization in yoghurt with varying levels of concentration. Further, prepared yoghurt was analyzed for chemical, sensorial and nutritional values.

2. Materials and methods

The present investigation was conducted at the Department of Food and Industrial Microbiology, College of Food Technology, Marathwada Agricultural University, Parbhani (MS) India.

2.1. Materials

The date palm fruit of two varieties viz. *Berhi* and *Safri,* were obtained from local market of Parbhani and Mumbai. . Cow milk of *red sindhi* breed was obtained from *Department of Animal Husbandry and Dairy Science*, MAU, Parbhani. Pure culture of S*treptococcus thermophillus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus* were obtained from National Chemical Laboratory, NCIM, Pune.

2.1.2. Composition MRS Media

Suspend all ingredients in 920 ml distilled water and heat to boiling to dissolve the medium completely. Sterilize the media in autoclave at 15 lbs pressure for 15 minutes. Proteose peptone (10g/L), yeast extract (5.0 g/L), beef extract (10g/L), dextrose (20g/L), Tween-80 (1.0g/L), ammonium citrate (2.0g/L), sodium acetate (5.0g/L), magnesium sulphate (0.1g/L), manganese sulphate (0.05g/L), dipotassium phosphate (2.0g/L).

2.1.3. Chemicals

Chemicals used in this investigation were of analytical grade. They were obtained from Department of Food And Industrial Microbiology, College of Agricultural Technology, MAU, Parbhani.

2.2. Methods

2.2.1. Physico-chemical analysis of date palm fruit

Date palm paste processed by different methods were analyzed for moisture content, protein, fat, reducing sugars, total soluble solids, mineral content, using standard chemicals and/or physical procedure [11]. Titratable acidity of the pulp was determined by titration against 0.1 N sodium hydroxide. The pH of Date palm paste was determined by using Perkin Elmer pH meter.

Table 1. *Physical characters of date palm (Phoenix dactylifera L.) fruit*

Physical parameter	***Berhi* fruit**	***Safri* fruit**
Number of fruit/kg	125	115
Weight of fruit (g)	8.00	8.70
Weight of seed (g)	0.86	1.40
Weight of flesh (g)	7.14	7.30
Fruit flesh %	89.25	83.91

Table 2. *Proximate analysis of date palm (Phoenix dactylifera L.) fruit*

Nr. Crt.	Parameters	***Berhi* fruit**	***Safri* fruit**
1.	Moisture (%)	14.7	15.3
2.	Fat (%)	0.4	1.8
3.	Protein (%)	2.03	2.60
4.	Carbohydrate (%)	78.02	77.53
5.	Ash (%)	2.8	2.6

Table 3. *Physico-chemical characters of date palm (Phoenix dactylifera L.) paste*

Nr. Crt.	Parameters	Varieties		Mean	S.D.	SE ±
		Berhi	Safri			
1.	Total soluble solids (^{0} bx)	58	56.2	57.1	1.272792	0.9
2.	pH	4.09	4.11	4.1	0.014142	0.01
3.	Acidity (%)	0.77	0.67	0.72	0.070711	0.05
4.	Moisture (%)	45.6	48.2	46.9	1.838478	1.3
5.	Fat (%)	0.5	1.4	0.95	0.636396	0.45
6.	Protein (%)	2.13	2.71	2.42	0.410122	0.29
7.	Carbohydrate (%)	87.64	86.23	86.935	0.997021	0.705
8.	Ash (%)	3.1	3.0	3.05	0.070711	0.05

Table 4. *Effect of varying concentrations of date paste on Settling time and synersis of yoghurt*

Treatments	Settling time (hr.)	Synersis (%)
C	8	20
B10	7.5	27.80
B15	6	24.60
B20	5	21.07
S10	7	22.43
S15	5.5	23.23
S20	4.5	21.20

Table 5. *Effect of addition of varying concentration of date paste on chemical composition of yoghurt*

Treatment (Yoghurt)	pH	Acidity (Lactic acid)	Moisture (%)	Fat (%)	Protein (%)	Total soluble solids(^{0}bx)
C	4.47	1.03	85.91	3.18	3.24	14.58
B10	4.54	0.97	69.37	3.27	3.25	24.2
B15	4.64	0.94	74.19	3.58	3.38	26
B20	4.69	0.87	74.94	3.71	3.42	27.8
S10	4.51	0.92	77.08	4.36	3.35	23
S15	4.58	0.89	77.18	4.61	3.43	24.2
S20	4.63	0.87	79.43	4.87	3.47	26.2
Mean	4.58	0.927143	76.87143	3.94	3.362857	23.71143
SD	0.078316	0.058513	5.092031	0.670721	0.089016	4.330387
S.E. $\pm$	0.029601	0.022116	1.924607	0.253509	0.033645	1.636732

Table 6. *Sensory analysis of yoghurt with different concentration of date paste*

Treatments	Appearance	Colour	Flavour	Taste	Texture	Overall acceptability
Control	7.5	7.0	7.2	7.5	7.4	7.3
B10	7.8	7.5	7.4	7.8	8.2	7.8
B15	6.8	7.1	7.6	7.2	7.3	7.1
B20	6.5	6.6	7.2	6.8	7.2	6.8
S10	7.7	7.4	8.3	8.6	8.0	8.0
S15	7.2	7.1	8.4	7.8	7.5	7.5
S20	6.8	7.0	7.2	7.1	7.4	7.0

2.2.2. Preparation of date paste

Ripe fruit with firm texture, uniform in size and maturity were used for the experiment. After cleaning the fruits, preliminary trials were conducted to standardize the methods of preparation of paste for this purpose. The optimum quality fruits of two different varieties were selected and manual separation of seed was carried out. Further, the fruit was cut into small pieces and grinder to obtained paste.

2.2.3. Preparation of yoghurt

Preparation of starter culture (mother culture)

The culture which was obtained from Subculturing i.e. S*treptococcus thermophillus* and *Lactobacillus delbrueckii* subsp.*Bulgaricus* was inoculated in conical flask containing sterilized 50ml cow milk in laminar flow. Then the milk was incubated at 42-45^0 C till the firm coagulum was formed.

Preparation of batch culture

Batch culture was prepared by inoculating 3 per cent of mother culture into 200 ml of sterilized cow milk and incubated at 42-45^0 C till firm coagulum was formed. The bulk culture was stored in refrigeration.

Raw Materials

The main raw material of yoghurt is fresh cow's milk. The milk contains about 13% TSS, including 3.5% fat. Skim milk powder (SMP) was used for fortification of the liquid milk.

Standardization/Mixing

Medium-fat yoghurt was produced, containing 1.5% milk fat. Centrifugal separation is used to remove about 2% of fat from the initial liquid milk and clarify the raw milk. The milk was fortified with milk proteins to produce a thick-body yoghurt product. Nonfat dry milk (NFDM) at the rate of 4% is usually added. Milk protein stabilizers, used to prevent whey separation (syneresis) of yoghurt, are added at the rate of about 0.3%.

Homogenization

The mixture of milk ingredients was homogenized in a high-pressure continuous homogenizer, operated at about 200 kg/cm^2 pressure and temperature of 55^{0}C. Homogenization retards fat separation (creaming) and improves the water-binding properties of milk proteins (casein).

Heat Treatment

The homogenized milk was heated to about 90^{0}C for 5 min for increasing the water-binding capacity of milk proteins and denaturation of the whey proteins. At the same time the milk mixture is pasteurized, i.e. all pathogenic and most spoilage bacteria are inactivated.

Fermentation

The heated milk mixture is cooled to about 45^{0}C and it is inoculated with a yoghurt culture. The yoghurt inoculums (wet a mixed culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in proportion of 3% of the milk mixture was incubated at 45^{o}C, after which a firm coagulum was formed. Set yoghurt was prepared by packaging the inoculated milk mixture into consumer cups and incubating the aseptically sealed packages at 45^{o}C for 3–4 hr.

Mixing of Yoghurt

The bulk-set yoghurt is cooled to 15^{o}C, the yoghurt containing date paste was prepared by gentle mixing of bulk yoghurt with about 10%, 15% and 20% of date palm fruit paste. The fruit product, containing yoghurt was stored at refrigeration temperature.

Packaging

Yoghurt or inoculated milk mixture was usually packaged in plastic cups and sealed with aluminum lids which operate under aseptic conditions.

Cooling/Storage

The sealed yoghurt cups are cooled and stored at about 5^0C. Refrigerated storage life was around 2–3 weeks.

2.3. Physical properties of fortified yoghurt

2.3.1. Setting time

The setting time of sample was recorded from the time of inoculation to just coagulum was formed and it was recorded in hours.

2.3.2. Determination of synersis

A sample of 100 gm centrifuged at 25^oC and whey was drained for 15 minutes. The weight of drained whey reported as the percentage of synersis.

2.3.3. Proximate composition

The Yoghurt s prepared from different levels of paste was analyzed for physio- chemical characteristics such as pH, moisture, protein and fat, total soluble solids and acidity has been determined as the procedures discussed above.

2.3.4. Sensory analysis

The sensory evaluation of yoghurt was carried out by a 10 trained panel member comprised of postgraduate students and academic staff members of the faculty who had some previous experience in sensory evaluation of fruit and vegetable products. The panel members were requested in measuring the terms identifying sensory characteristics and in use of the score. Judgments were made through rating products on a 9 point Hedonic scale with corresponding descriptive terms ranging from 9 'like extremely' to 1 'dislike extremely'.

2.4. Statistical analysis

The analysis of variance of the data obtained was done by using Completely Randomized Design (CRD) for different treatments. The analysis of variance revealed at significance of $P < 0.05$ level, S.E. and C.D. at 5 % level is mentioned wherever required.

3. Result and discussion

In this present investigation attempts have been made judge the suitability of date palm paste variety with varying levels for yoghurt fortification. Yoghurt is made by combining different levels of paste after fermentation. The product obtained, was evaluated for its sensory and physico-chemical properties. In this investigation there are in all seven treatments including control and the combinations are summarized below:

Sample	Variety /Concentration fruit pulp
C	Control without date paste
B10	Yoghurt with 10 % date paste of Berhi variety
B15	Yoghurt with 15 % date paste of Berhi variety
B20	Yoghurt with 20 % date paste of Berhi variety
S10	Yoghurt with 10 % date paste of Safri variety
S15	Yoghurt with 15 % date paste of Safri variety
S20	Yoghurt with 20 % date paste of Safri variety

3.1. Physical characterization of date palm fruits

The date palm fruit were cylindrical shaped and dark red in colour. The physical

characteristics of date palm fruits used in the present investigation are given in Table 1.

The data depicted in Table 1 revealed that the average one kilogram of *Berhi* fruit contained about 125 fruits and 115 *Safri* variety of date palm respectively. The *Berhi* date palm fruit contained about on an average 89.25% flesh and *Safri* variety contained about 83.91% flesh and 11.75% and 17.09% pit respectively. Similar results were obtained as stated by Jahromi *et al* [12].

3.1.1. Proximate composition of date palm fruit

The data pertaining to proximate analysis of Berhi and Safri date palm fruits was recorded and presented in Table 2.

The purpose of finding the chemical composition was to study the effect of date composition on physiochemical and sensory quality of yoghurt. The data in Table 2 revealed that there was significant difference in two categories in respect of various parameters of proximate analysis. As regarded moisture percentage in fruit of two categories it is clear from the data that *Safri* fruit found to contain significantly more moisture (15.30%) than *Berhi* variety (14.7%).

The *Safri* fruit contained more fat (1.8 per cent) and protein (2.60 per cent) as compared to other category. In respect of carbohydrate the *Berhi* date fruit contains more carbohydrate to *Safri* date fruit, as it contained carbohydrate (78.2 per cent). With regards to ash it is evident that *Safri* fruit (2.6%) recorded low ash then *Berhi* fruit (2.8 %). But the difference in the fat content is quite significant. A difference in chemical composition of varieties is obvious as the processing of these fruits differ [13].

3.1.2. Physico-chemical characters of date palm paste

As depicted in Table 3, the *Berhi* fruit paste contained higher total soluble solids (58^{o}Bx), carbohydrate (87.62 per cent) and acidity (0.77) while Safri variety reported to be superior in terms of protein, fat, and moisture content.

The difference in chemical composition of paste of different varieties is obvious as the processing variations and difference in their fruit composition.

3.2. Physical properties of date paste fortified yoghurt

3.2.1. Settling time

Table 4 showed that the use of date palm paste different concentration affected significantly on setting time of fortified cow milk yoghurt. Setting time of yoghurt reduced with increased in the concentration of the fortification.

The minimum setting time was observed in sample S20 (4.5hr) among all samples, while maximum settling time was reported in control sample (8.0hr). Reduction in setting time of fortified yoghurt of present investigation is due to the increase in concentration of date palm paste. Hence, it is clear from the above readings that settling time is inversely related to levels of date paste incorporation.

Londsted [14] reported the coagulation time of 5 to 6 hrs, whereas Rao *et al.* (1982) reported the coagulation time of yoghurt in the range of 5 to 7 hrs with different culture combination. The interaction of pectin as protective hydrocolloid associated with casein in low pH milk product with effect of two different commercial pectins on setting time of yoghurt. The present results are in close confirmation with these findings.

3.2.2. Synersis

The data in Table 4 revealed that the yield of synersis influenced significantly by increase in acidity of product. The yoghurt containing 10 per cent paste of *Berhi* date sample 'B10' had significantly higher synersis. This may be due to higher acidity

resulting in separation of whey and total solids. The minimum synersis was found in B20 and S20 21.07 and 21.20 per cent respectively.

3.2.3. Chemical composition of date paste fortified yoghurt

pH

The pH values of samples varied from 4.51 to 4.69. Table 5 shows the pH content of sample 'B20' (4.69) was maximum as compared to other samples. Minimum pH was recorded in sample 'S10' (4.51) with 10 per cent paste of *Safri* date variety.

The pH of yoghurt found to increase with increase in concentration of both varieties, while pH reading were higher in *Behri* variety compared to *Safri*. This may be due to the more carbohydrate content of the *Berhi* variety being converted into acid in fermentation process. Zekai and Erdoğan [15] reported pH values of yoghurt in the similar range as obtained during present study.

Acidity

It may observe from Table 5 that the acidity of fortified yoghurt was affected significantly due to addition of date palm paste. The acidity of fortified yoghurt found to increase with increase in concentration of date paste. O' Neil *et al.* [16] observed an increase in acidity with increase in concentration.

Moisture

The sample 'S20' has maximum moisture (77.18 per cent) treated samples followed by 'S15' (77.08). Sample 'B10' (69.37 per cent) was with minimum moisture content among the samples of fortified yoghurt samples. In case of *Safri* variety the decrease in moisture content is more significant. These results are similar to the results obtained by Hashim [17].

Fat

There is significant difference in the fat content of the yoghurt containing two different variety of date paste. It has seen that *Safri* variety imparted more fat than the *Berhi* variety as the date paste of *Safri* variety was containing more fat than the *Berhi* variety. While, surprisingly S15 contained higher fat per cent i.e. 4.61 compared to S20 (4.87).

Protein

Safri variety found to impart more protein content *Berhi* variety as shown in Table 5. Protein content ranges from 3.45 to 3.47 per cent. Zekai and Erdoğan [15] reported protein content of yoghurt in the same range obtained in present study.

Total soluble solids

Total soluble solids of yoghurt varied from 14.58 (control) to 27.8 (B20). From Table 5 it was recorded that *Safri* variety found to contain lower total soluble solids compared to *Behri* variety. Among treated samples, lowest TSS was observed in yoghurt containing 10 per cent paste of *safri* variety. The total soluble solid found to increase with increase in date paste concentration in yoghurt. But the total soluble solid is more in the case of *Berhi* variety than the *Safri* date variety.

3.2.4. Sensory evaluation of yoghurt fortified with date paste added after fermentation

Appearance

The sample S10 containing 10 per cent of *Safri* date palm paste found to have highest score to control sample (plane yoghurt) followed by B10 containing 10 per cent of behri paste which is similar in appearance as that of control sample. It is learned that at lower concentration, date palm paste improves the appearance property, however further increase in concentration resulted in decrease in

appearance of product. This may be due to reduction in water holding capacity of the curd mass as it affects the protein interaction during coagulation process, resulting into more yield of synersis.

Colour

The difference in colour of the fortified yoghurt at lower concentration, were highly significant due to addition of different concentration date palm paste of different variety. Among the all sample, the yoghurt having B10 with 10 per cent concentration of paste of *Behri* date fruit was scored maximum score for colour i.e. 7.5. Control sample (Plain yoghurt) was not superior to other fortified yoghurts. It means that as compared to plain yoghurt, colour was improved due to addition of date paste. However, further increase in concentration beyond 10 per cent resulted in decrease in color acceptability even if the intensity of color was increase. It could be concluded from color observation that, consumer prefers light colored yogurt, whereas darkly colored yogurt reduces acceptability. *Behri* varieties have better color properties than *Safri*.

Flavour

Flavour of fortified yoghurt was significantly influenced by date paste incorporation. For flavour, the sample 15 per cent paste of *Safri* found to be superior amongst all the other samples. Further increase in concentration reduced flavour acceptability. This may be due to further increase in concentration resulted in increasing the alcoholic aroma and acidic taste of yoghurt. So, the minimum concentration should be preferred for the maximum flavour score.

Taste

The taste of fortified yoghurt was influenced significantly due to addition of date paste. From the table, it was observed that sample 'S10' (8.6) with 10 per cent paste of *Safri* variety were scored maximum in taste. The 10 per cent concentration was more preferred than 15 and 20 per cent paste of both date variety paste in yoghurt. The taste of sample 'S10' was liked extremely to the judging panels. As it is natural sweetener it enhanced the taste of yoghurt. Fortified S10 yoghurt was superior to control sample Plain yoghurt). It means that as compared to plain yoghurt, taste was improved due to addition of date paste prior fermentation of milk.

Texture

It is noted from Table-6 that the texture of fortified yoghurt was affected significantly due to addition of different concentration and variety date palm paste. It is revealed that, higher level of concentration of fruit paste and reduced the score for texture. Texture was generally affected due to separation of whey at high level of paste due to production of acids, to give the reduced coagulation and formation of soft and loose textured curd.

The score of fortified yoghurt was good as compared to control sample. With respect to the highest score of (8.2 per cent) was obtained to sample 'B10' followed by sample 'S10' 8.0 and 'S15' with score 7.5.

Overall acceptability

As shown in Table 10, B10 and S10 sample were superior to control sample (Plain yoghurt). It means that overall acceptability was improved due to addition of date paste. Among the all the treatments, S10 having the maximum score i.e.8.0.

4. Conclusion

Present investigation was undertaken to study the effect of date paste of two variety viz. *Berhi* and *Safri* and physiochemical properties and organoleptic properties of yoghurt.

Seven samples were prepared by different concentration of date paste with one control. Date palm variety i.e. *Berhi* was found to contains higher TSS, mineral and fibre than *Safri* variety. It also has been found that the per cent paste obtained from one kg of *Berhi* variety date was more than *Safri* variety. It was found that increase in concentration of date paste in yoghurt decreases setting time irrespective of variety. With respect to synersis of yoghurt, synersis decrease with increasing concentration of paste. The acidity, moisture and total soluble solids was found to increase with increasing concentration of date paste in case of sample. Organoleptic evaluation with colour, appearance, flavour, taste and overall acceptability showed highly significant effect due to addition of date paste. Yoghurt containing 10 per cent of date paste found to be superior compared to control in case of both variety and treatment.

It is concluded from the present investigation that the use of date paste improved the qualities of the fortified cow milk yoghurt at lower concentration up to 10 per cent. While with respect to varieties, *Safri* was more suitable than *Behri* variety for yoghurt fortification. Hence, it is concluded that addition of 10 per cent *Safri* date paste in yoghurt improves the organoleptic as well and physico-chemical properties of yoghurt.

5. References

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EFFECT OF HYDROCOLLOIDS ON THE QUALITY CHARACTERISTICS OF TOMATO KETCHUP

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ABSTRACT

The effect of two hydrocolloids guar gum and xanthan gum at varying concentration was studied on the yield, viscosity, serum loss and sensory characteristics of tomato ketchup. The yield of tomato ketchup increased substantially from 33% to 48.05% upon the addition of hydrocolloids. The increase in yield followed the order control < xanthan gum (0.25%) < guar gum (0.25%) < guar gum (0.50%) < xanthan gum (0.50%). All combinations resulted in reduced serum loss in the tomato ketchup. Guar gum at 0.5% caused maximum decrease in serum loss. The viscosity of the ketchup increased with the increase in the concentration of the two hydrocolloids. Sensory analysis results revealed that all the tomato ketchups prepared in the study were acceptable and the one with 0.50% guar gum was preferred the most by the penal members. It was concluded that 0.50% guar gum was the best thickener among the various combinations of hydrocolloids studied.

Keywords: *guar gum, xanthan gum, serum loss, viscosity, sensory, tomato ketchup*

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1. Introduction

Tomato is one of the most important vegetable products mainly marketed in a processed form i.e. pastes, concentrates or ketchup [1]. Ketchup is a descriptive term for a number of different products, which consist of various pulps, strained and seasoned fruits; the variety made from tomatoes being the most popular condiment. Tomato ketchup is a heterogeneous, spiced product, produced basically from either cold or hot extracted tomatoes; or directly from concentrates, purees or tomato paste [2]. Ketchup is a popular condiment, usually made with ripened tomatoes. The basic ingredients in modern ketchup are tomatoes, vinegar, sugar, salt, spices and thickening agent[3]. Thickening agents are natural or chemically modified carbohydrates that absorb some of the water present in the food, thereby making the food thicker [2]. Consumers prefer thick products, therefore, tomato ketchup is now prepared with the addition of thickeners [4]. Hydrocolloids are water-soluble, high molecular weight polysaccharides that serve a variety of functions in food systems including enhancing viscosity, creating gel-structures, film formation, control of crystallization, inhibition of syneresis, improving texture, encapsulation of flavours and lengthening the physical stability [5,6,7,8]. Hydrocolloids can be added to improve consistency and decrease the serum loss of the ketchup [9,10]. The main objective of the present study was to analyze the effect of thickeners on quality characteristics like sensory and viscosity of tomato ketchup at different level.

2. Materials and methods

Fresh ripe tomatoes were obtained from the local market, Hisar (India). The tomatoes were washed, crushed and then blanched. They were then passed through a laboratory sieve to get tomato pulp. The tomato ketchup was prepared [9] using the ingredients shown in Table 1. The tomato

pulp having a total soluble solids (TSS) content of 4.5% was put in an open pan and the spices were wrapped in a muslin cloth and dipped into the juice. Onion, ginger and garlic were also pulped and added to the juice. The ketchup was heated on a low flame with constant stirring till a final TSS of 28% was obtained. Then sodium benzoate (750 ppm) was added as a preservative. TSS of tomato juice and ketchup was measured with the help of Hand Refractometer. Two different hydrocolloids namely guar gum and xanthan gum were added to the ketchup at different levels of 0.25 % and 0.5% and combination of guar and xanthan gum at 0.50%, just before the end point. The hydrocolloids were pre-blended with the sugar and salt and then added to the ketchup during the final stages of cooking. The ketchup was filled hot in glass bottles, sealed with crown corks and stored.

Table 1. *Recipe used for the preparation of tomato ketchup*

Ingredients	Quantity used
Tomato juice	1 Kg
Sugar	80 g
Salt	10 g
Onions	3.0 g
Ginger	6.0 g
Garlic	6.0 g
Cloves (headless)	0.3 g
Cinnamon	0.3 g
Red chili powder	1.5 g
Black pepper powder	0.002 g
Acetic acid (99.5%)	2.5 mL

2.1. Analysis of tomato ketchup

Moisture content of the ketchups was determined by AOAC [11]. Total soluble solid (TSS) was measured by Hand Refractometer. The ability of the gums to hold water in the ketchup was also measured. Tomato ketchup (20 g) was taken in a centrifuge tube and then centrifuged at 5000 rpm for 10 min. The supernatant was discarded and the remaining ketchup was weighed. Serum loss was calculated according to the following equation [9]:

$$\text{Serum Loss},\% = \frac{\text{weight of the serum removed}}{\text{weight of ketchup}} \cdot 100 \quad (1)$$

Reduction in serum loss in the ketchup samples was also calculated according to the following equation:

$$\%\text{Reduction in serum loss} = \frac{SR_{control} - SR_{sample}}{SR_{control}} 100 \quad (2)$$

where $SR_{control}$ and SR_{sample} is the serum loss in control ketchup and serum loss in sample ketchup, respectively.

Viscosity analysis of the ketchup samples were carried out with Brookfield Viscometer (RV model) at 25ºC and spindle no. 5 at 20 rpm. Yield of the ketchups was also calculated. All the results were obtained using samples from three different batches.

2.2. Sensory evaluation of tomato ketchup

The sensory attributes like taste, color, appearance, aroma, texture and overall acceptability of the product were determined using a taste panel consisting of 10 panelists. The ratings were on a 9 point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely).

2.3. Statistical analysis

The experimental data collected was analyzed for significant differences with the help of analysis of variance (ANOVA) conducted using SPSS 16.0 software.

3. Results and discussions

Chemical composition of the tomato pulp used was investigated and has been presented in the Table 2.

Table 2. *Chemical composition of the tomato pulp*

Component	Proportion, %
Moisture	92.88
Ash	0.0414
Ascorbic acid	0.024
Acidity	0.418
Total soluble solid	4.5
Reducing sugar	2.687
Dry matter	7.12

Effect of addition of guar gum (GG), xanthan gum (XG) and combination blend of both gums was studied on various quality parameters of tomato ketchup. The results have been presented in Table 3.

3.1. Effect of hydrocolloids on moisture, ash content and acidity of ketchup

The moisture content of the different ketchup samples increased with the increase in concentration of the hydrocolloid used compared to the control. The thickening agents act as water-binding materials [12], which hold water and prevent moisture removal during the cooking process. Thus the addition of hydrocolloids increased the consistency of ketchup, because the moisture did not vaporized easily. Ash content decreased from 0.88 in control ketchup to 0.71 in 0.5% guar + xanthan gum ketchup. The ash content decreased with the increase in concentration of the hydrocolloids. The acidity of tomato ketchup also gradually decreased upon the addition of increasing level of hydrocolloids. High moisture content of the treated tomato ketchup samples shows decline in the ash and acidity value of tomato ketchup on addition of hydrocolloids may probably due to the dilution effect. Addition of hydrocolloids substantially increases the water binding in tomato ketchup through hydrogen bonding which increases the moisture content in final product. Hence similar weight of treated tomato ketchup sample shows lesser amounts of ash. Similarly acidity of treated samples is also diluted.

3.2. Effect of hydrocolloids on yield, viscosity and serum loss of tomato ketchup

The yield of different tomato ketchup is presented in Table III. The yield of different tomato ketchups increased from 33.33% to 48.05% upon the addition of the hydrocolloids. It was also found that 0.5% level of addition of guar + xanthan gum gave the highest yield among all samples. The yield increased in the order- control<0.25% guar gum< 0.25% xanthan gum< 0.50% guar gum< 0.50% xanthan gum< 0.5% guar + xanthan gum. It may probably due to water binding capacity of these gums. Similar results were obtained by Sahin and Feramuz (2004), who reported that addition of hydrocolloids (CMC, guar gum, xanthan gum) increased the amount of tomato paste[2].
The viscosity of the ketchups increased with the increase in concentration of hydrocolloids, the increase being more prominent for guar gum samples. The viscosity increased significantly from 15200 to 18400 cPs [1cP = 0.001 Pa.s] upon addition of hydrocolloids. Addition of hydrocolloids increases the viscosity of any product containing water system by entrapping the water molecule in the hydrocolloid gel matrix. Thus, hydrocolloids improve the consistency of product by water phase management.
The serum loss (syneresis) indicates the ability of tomato ketchup to hold water. The statistical analysis in Table 3 revealed a highly significant effect of hydrocolloid concentration on serum loss of tomato ketchup. Addition of hydrocolloids led to a decrease in the serum loss. There was no serum loss in the tomato ketchup to which 0.5% guar gum was added. Guar gum, xanthan gum and guar gum + xanthan gum (50:50) decreased the serum loss by 99.65%, 96.51% and 98.25%.

Table 3. *Effect of hydrocolloids on different parameters of tomato ketchup*

Ketchup	Moisture, %	Ash, %	Acidity, %	Yield, %	Viscosity, (cPs)	Serum loss, %	Resuding in serum loss, %
Control	63.57±0.46a	0.88±0.04e	1.32±0.06f	33.33±1.42a	15200±20.0a	2.87±0.32f	-
S_1	68.47±0.40c	0.84±0.02d	1.27±0.05e	38.41±1.44b	17600±14.0c	0.49±0.08e	82.84a
S_2	72.44±0.38d	0.77±0.02c	1.18±0.04c	42.26±1.28d	18200±30.0e	0.01±0.00a	99.65e
S_3	67.53±0.52b	0.84±0.01d	1.25±0.02d	39.14±1.34c	17200±18.0b	0.37±0.03d	87.12b
S_4	73.64±0.24e	0.75±0.04b	1.16±0.03b	46.05±1.24e	17800±24.0d	0.10±0.01c	96.51c
S_5	79.56±0.32f	0.71±0.05a	0.96±0.03a	48.05±1.34f	18400±20.0f	0.05±0.01b	98.25d

[a]The values are mean ± S.D. of determinations made in triplicates. Values followed by different letters are significantly different at $P< 0.05$. S_1: 0.25 % guar gum, S_2:0.50% guar gum, S_3:0.25% xanthan gum, S_4:0.50% xanthan gum, S_5:0.50% (guar gum+ xanthan gum, 50:50)

Table 4. *Sensory evaluation of tomato ketchup*

Ketchup	Color	Appearance	Aroma	Texture	Taste
Control	6.68±0.42c	7.21±0.36c	7.20±0.44d	6.82±0.38d	6.63±0.28d
S_1	7.18±0.52d	7.34±0.48c	6.71±0.35c	6.94±0.50e	6.91±0.26e
S_2	7.53±0.34e	7.63±0.38d	7.26±0.26d	7.35±0.46f	7.58±0.24f
S_3	6.33±0.58b	6.22±0.42b	6.30±0.44b	6.28±0.50c	6.33±0.32c
S_4	6.18±0.42a	5.94±0.24a	5.89±0.36a	5.58±0.34a	6.08±0.46a
S_5	6.42±0.36b	6.21±0.38b	6.36±0.44b	6.08±0.42b	6.21±0.26b

[a]The values are mean ± S.D. of determinations made in triplicates. Values followed by different letters are significantly different at $P< 0.05$. S_1: 0.25 % guar gum, S_2:0.50% guar gum, S_3:0.25% xanthan gum, S_4:0.50% xanthan gum, S_5:0.50% (guar gum+ xanthan gum, 50:50)

This indicated that both xanthan gum and guar gums have excellent water holding capacity and are excellent in preventing the occurrence of syneresis in tomato ketchup.

3.3. Sensory evaluation of tomato ketchup

Preference by consumer is strongly desired in successful promotion of food products in market. Sensory evaluation results are shown in Table 4. Results obtained from sensory tests showed that all tomato ketchup samples were ranked acceptable by the panel member. Significant differences were reported among the different tomato ketchups prepared in the study in the sensory evaluation results. The ketchup samples containing guar gum as thickener were liked more by the panel member than samples containing xanthan gum and blend of guar and xanthan gum. Moreover, ketchup samples containing 0.50% concentration of guar gum was preferred in each aspect including color, appearance, aroma, texture and taste.

4. Conclusions

The study was designed to assess the effect of guar gum and xanthan gum on the quality of tomato ketchup. Moisture content, yield and viscosity of tomato ketchup increased on addition of guar gum as well as xanthan gum. Ash content, acidity and serum loss decreased substantially upon addition of guar and xanthan gum. Sensory results showed that guar gum was more suitable thickener for tomato ketchup at 0.5% concentration

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CONCENTRATION OF ANTIBIOTICS RESIDUES IN FARMED TILAPIA AND THEIR RELATIONSHIP TO RESISTANT *AEROMONAS* STRAINS

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ABSTRACT

Three groups of fresh tilapia, *Oreochromis niloticus*, were randomly collected from farms at Port-Said Governorate, Egypt for qualitative and quantitative detection of antibiotic residues by microbiological assay method and HPLC. The sensitivity of isolated Aeromonas strains for oxytetracycline was also evaluated. The detections of antibiotic residues in tilapia samples were found in a percentage of 100% (60), 80% (48) and 0.00% (0.00) for group I, II and III respectively. The detected antibiotics were from the tetracycline and ß–lactam groups. The mean concentrations of the oxytetracycline residual level in tilapia muscle assessed were 319.92 ± 12.18, 142.14 ± 4.86 and 75.49 ± 1.66 ppb for group I, II and III respectively. The isolated strains of Aeromonas species in the examined samples were various among the examined groups and phenotypic identified as A. caviae, A. scubertii, A. hydrophila, A. trota and A. sobria. The incidence of oxytetracycline resistance Aeromonas strains in group I and II were 100% for each, while 35.71% of the isolated strains in the group III were resistant to oxytetracycline. Antibiotics residues are seemed to quality defects in farmed tilapia that facing the challenges of the fish exporting in Egypt. Application of good manufacture practices and Hazards Analysis Critical Control System (HACCP) in tilapia farms must be done for improving the antibiotics residual problem in fish muscles.

Keywords*: Fish, microbiological assay, oxytetracycline, Bacteria*.

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1. Introduction

Aquaculture has been considered one of the fastest growing segments of the global food production sector in recent decades [1]. In comparison to other farmed animals, fish are generally very efficient converters of feedstuffs into high quality protein [2]. Tilapias, *Oreochromis niloticus*, have become the third most important fish in aquaculture that cultured in many tropical and subtropical regions. It is characterizes by high yields in a short period on relatively low nutritional inputs and high palatability [2].

Member of the genus *Aeromonas* are ubiquitous in most aquatic environments through the world [3]. Several strains have been implicated in fish disease as *Aeromonas hydrophila, A. Sobria, A. allosacchharophila, A. Salmonicida and A. veronii* [4]. *Aeromonas hydrophila* is one of the most important agents of the outbreaks in fresh water fish, in which skin ulcer, hemorrhage and necrosis of the visceral organs are the major symptoms of Aeromonad septicemia [5]. Diseases outbreaks due to *Aeromonas* species are responsible for high economic losses in both intensively and extensively cultured fish as well as in wild fish [6] as a result of high mortality and deterioration of product quality [7, 8]. Some strain of *Aeromonas* species such as *Aeromonas hydrophila, A. veronii, A. jandaei, A. trota* and *A. schubertii* have been emerged as opportunistic human pathogens [9] which responsible for foodborne gastroenteritis, pneumonia, peritonitis, endocarditis, meningitis, septicaemia, urinary tract infection and wound and skin infection specially in compromised patients [10, 11].

The potential for disease problems associated with intensive fish culture would increase the probability of the use of antimicrobial drugs to treat a variety of bacterial infection [12] beside promoting the

fast growth of the fish [13]. Tetracyclines such as tetracycline, chlortetracycline, doxytetracycline, and oxytetracycline are probably the most widely used therapeutic antibiotics in human and food-producing animals because of their broad-spectrum activities and cost effectiveness [14]. The number of antibiotics used in fish farming is restricted, but oxytetracycline is probably the most frequently used therapeutic antibiotics in fish farms to treat the bacterial infection and limits the fish mortality, particularly in controlling of *Aeromonas hydrophila* and *A. Salmonicida* [15, 16].

The abuse used of the antibacterial drugs in the ecosystem specially, at farm level can be accumulating in Tilapia till reach above the standard permissible limits consequently increase the potential hazards for consumer's health [13]. Antibacterial drugs have the potential to gradually accumulate in the body of the consumers causing certain organs or system malfunction [13] such as disorders in the intestinal flora, hypersensitivity reaction, chronic nephrotoxicity, hepatotoxicity, skin hyperpigmentation in the sun exposed areas, hypouricemia, hypokalemia, proximal and distal renal tubular acidosis, poor fetal development, and secondary tooth discoloration of young children [17]. In addition, the transmission of the resistance gene from the pathogenic and non pathogenic bacteria and in between them to the human through the food chain results in treatment-resistant illness in human [18, 19, 20].

Therefore, this study was conducted to evaluate the safety of farmed *Oreochromis niloticus* for human consumption through the qualitative and quantitative detection of antibiotics residue levels in fish muscle as well as examine the isolated *Aeromonas species* for oxytetracycline susceptibilities.

2. Material and Methods

2.1. Samples collection

Three different groups of farmed tilapia, *Oreochromis niloticus,* previously known be treated by oxytetracycline were collected during summer season from fish farms at Port-Said Governorate, Egypt. Dead fish was discard and a total of 180 sample, 60 for each group, as soon as they cough, were separately placed into sealed sterile plastic bag, thoroughly identified and delivered to the laboratory in a refrigerated container for qualitative and quantitative detection of antibiotics residues with the investigation of the *Aeromonas* isolates for oxytetracycline resistance.

2.2. Detection of Antibiotics residues

Antibiotics residues of fish samples were carried out by Agar Disk Diffusion Method according to Myllyniemi [21]. Three flasks of Muller Hinton agar media were prepared according to the manufacturer's instructions and adjusted to pH 6, 7.2 and 8 then poured in Petri-dish of 90 mm diameter at 45-50°C. For each sample, three solidified Muller Hinton agar plates were inoculated with *Bacillus subtilis* as a test strain. Trimethoprim was incorporated into the pH 7.2 media (plate 2) with a concentration of 0.005 mg/ml to enhance the sensitivity towards sulfonamides residues. The appropriates discs of standard penicillin, sulfamethazine and dihydrostreptomycin were placed in the center of plates 1, 2, and 3 respectively. Fish sample was prepared by cutting and slicing the muscle into 2 mm thickness using cork borer. Six pieces of different muscle samples and one negative control paper disc were placed in each plate at a distance of 10 mm from the plate's edge, and then incubated at 30°C for 18 hr. The interpretation of the results depending up on the diameter of the inhibition zone around the samples where, the complete inhibition of the growth in an annular zone around the

muscle disc samples measured ≥2 mm wide indicates positive results and detection of certain antibiotic. On the other hand, the diameter of inhibitory zone around the disc samples measured ≥1 to < 2 mm indicates suspicion result while a measured <1 mm of the diameter indicates negative results and no detection of antibiotic. Plates with pH 6 is to detect in particular beta-lactam and tetracyclines residues while that with pH 7.2 and pH 8 are to detect particular sulfonamides and amninoglycosides residues respectively.

2.3. Quantitative assessment of oxytetracycline

Fish samples were individually conducted to analyzed, identified and quantified determination of oxytetracycline residues by high performance liquid chromatography (*Agilent 1200 series-Germany*) according to AOAC [22].

2.4. Isolation of Aeromonas species

A representative 10 grams of fish muscle were aseptically transferred to a stomacher bag (*Seward medical, London, UK*) containing 90 ml of sterile trypticase soy broth (TSB, Difco) added with 20 µgmL/L of ampicillin (TSBA) and homogenized for 60 second with stomacher (Lab. *Circulator Stomacher 400, Seward Medical, London UK*) at room temperature, then incubated at 28ºC for 24 h. Then tenfold serial dilution was prepared using sterile trypticase soy broth (TSB, Difco) with 20 µgmL/L of ampicillin (TSBA) till dilution 10^3. Spreading 0.1 mL of each dilution (10^{-1} - 10^{-3}) onto the surface of two GSPA (*Pseudomonas Aeromonas* selective agar) plates and re-incubating at 28ºC for 24 h. From each plate 2 to 5 of typical colonies (yellow colonies of 2-3 mm, surrounded by a yellow-zone) were transferred to trypticase soy agar (Difco) for biochemical identification tests according APHA [23].

2.5. Sensitivity of Aeromonas to Oxytetracycline

A purified and phenotypic identified *Aeromonas* isolates from *Oreochromis niloticus* fish samples were tested in vitro for oxytetracycline (30 µg) susceptibilities according to CLSI [24].

2.6. Statistical analysis

Minimum, maximum, mean, standard error and standard deviation of means were used to describe data. One-Way ANOA test was used to compare the mean of the oxytetracycline residual level in the three groups of *Oreochromis niloticus* fish samples. These tests were analyzed using the Statistical Package for Social Scientists (SPSS) for windows 16.0 (*SPSS Inc., Chicago, IL, and USA*).

3. Results and discussion

The qualitative detection of antibiotic residues in the muscle of tilapia samples were summarized in Tables 1, 2 and 3. The positive detection of antibiotic residues in the examined samples was detected in group I and II in a percentage of 100% (60) and 80% (48) respectively. Fish of group III showed negative detection of antibiotic residues by 33.33 % (20), while 20% (12) and 66.67% (40) of group II and III were considered as suspicion detection for antibiotic residues respectively. These results were agreed with the results recorded by Nusbaum and Shotts [25] who recorded that oxytetracycline was the most widely used therapeutic antibiotics in fish farms. Different levels of antibiotics residues were recorded by Killing [26]. It may be attributed to the variation in the duration of feed application and the withdrawal period, whereas the inhibition zones increased according to fish fed with pellets containing antibiotics. High dose of oxytetracycline leads to more rapid excretion than low dose consequently affect the diameter of inhibition zones [27].

Table 1. *Results of Qualitative Detection of Antibiotic Residues in the Muscle of Tilapia (n= 180)*

	Group I (n=60)						**Group II (n=60)**						**Group III (n=60)**					
	Plate 1		**Plate 2**		**Plate 3**		**Plate 1**		**Plate 2**		**Plate 3**		**Plate 1**		**Plate 2**		**Plate 3**	
	***B. subtilis* pH 6**		***B. subtilis* pH 7.2**		***B. subtilis* pH 8**		***B. subtilis* pH 6**		***B. subtilis* pH 7.2**		***B. subtilis* pH 8**		***B. subtilis* pH 6**		***B. subtilis* pH 7.2**		***B. subtilis* pH 8**	
	No.	%.	No.	%.	No.	%.	No.	%.	No.	%.	No.	%.	No.	%.	No.	%.	No.	%.
<1 mm	-----	-----	60	100	60	100	-----	-----	60	100	60	100	20	33.33	60	100	60	100
≥1 to < 2 mm	-----	-----	-----	-----	-----	-----	12	20.00	-----	-----	-----	-----	40	66.67	-----	-----	-----	-----
2 mm	-----	-----	-----	-----	-----	-----	16	26.67	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
3 mm	-----	-----	-----	-----	-----	-----	24	40.00	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
4 mm	-----	-----	-----	-----	-----	-----	8	13.33	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
5 mm	8	13.33	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
6 mm	20	33.33	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
7 mm	20	33.33	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
8 mm	12	20.00	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Total	60	100	60	100	60	100	60	100	60	100	60	100	60	100	60	100	60	100

≥2 mm wide inhibition zone → indicates detection of antibiotic

≥1 to < 2 mm inhibition zone → indicates suspicion detection of antibiotic

<1 mm inhibition zone or no inhibition zone → indicates no detection of antibiotic or under the detection limits.

Table 2. *Frequency Distribution of Inhibitory Zone Diameter among the Microbiological Assay of Tilapia Samples (n= 180)*

	plate 1			**plate 2**			**plate 3**			**Total**
Inhibitory zone diameter	**<1 mm**	**≥1-< 2 mm**	**≥2 mm**	**<1 mm**	**≥1-< 2 mm**	**≥2 mm**	**<1 mm**	**≥1-< 2 mm**	**≥2 mm**	
Group I	0.00	0.00	60	60	0.00	0.00	60	0.00	0.00	180
Group II	0.00	12	48	60	0.00	0.00	60	0.00	0.00	180
Group III	20	40	0.00	60	0.00	0.00	60	0.00	0.00	180
Total	20	52	108	180	0.00	0.00	180	0.00	0.00	540

Table 3. *Number of Positive and Negatives Tilapia Samples Based on Antibiotic Residues*

Group	Positive detection (≥2 mm)		Suspicion detection (≥1 to < 2 mm)		Negative detection (<1 mm)	
	No.	%	No.	%	No.	%
I	60	100	0.00	0.00	0.00	0.00
II	48	80	12	20	0.00	0.00
III	0.00	0.00	40	66.67	20	33.33

Table 4. *Statistical Result of Oxytetracycline Residues (ppb) Recovered from Tilapia Samples (No. =180*)

Groups		I	II	III
Number of samples	**No.**	60	60	60
	%	100	100	100
Non detected samples	**No.**	0.00	4	28
	%	0.00	6.67	46.67
Detected samples	**No**	60	56	32
	%	100	93.33	53.33
Oxytetracycline residues (ppb)	**Min.**	205.66	75.89	60.08
	Max.	486.93	195.88	90.21
	Mean	319.92	142.14	75.49
	S. E.	12.18	4.86	1.66

Table 5. *Incidence of Oxytetracycline Residual (ppb) Levels in Comparison with the Standard Limits for Tilapia (100 ppb*)

		Group I (n=60)		Group II (n=60)		Group III (n=60)	
		No.	%	No.	%	No.	%
Acceptable	**Detected**	0.00	0.00	8	13.33	28	46.67
	Not detected (<50 mm)	0.00	0.00	4	6.67	32	53.33
Not Acceptable		60	100	48	80.00	0.00	0.00
Total		60	100	60	100	60	100

Table 6. *Correlation between Examined Fish Groups for the Different Levels of Oxytetracycline Residues*

	Group I (n=60)	**Group II (n=60)**	**Group III (n=60)**
Group I (n=60)	----------	.000 (**)	.000 (**)
Group II (n=60)	.000 (**)	----------	.000 (**)
Group III (n=60)	.000 (**)	.000 (**)	----------

(*) = Significant relationship at 0.05 (95%).
(**) = Highly significant relationship at 0.01 (99%).

Table 7. *Results of the Inhibitory Zone of the isolated Aeromonas species Recovered from Tilapia Samples Against Oxytetracycline Disc (30 µg)*

Isolated strain	**Zone diameter**								
	Group I (n=60)			**Group II (n=60)**			**Group III (n=60)**		
	R (≤ 14mm)	**I (15-18 mm)**	**S (≥ 19 mm)**	**R (≤ 14mm)**	**I (15-18 mm)**	**S (≥ 19 mm)**	**R (≤ 14mm)**	**I (15-18 mm)**	**S (≥ 19 mm)**
Aeromonas caviae	4 mm	----------	----------	8 mm	----------	----------	14 mm	----------	----------
Aeromonas caviae	5 mm	----------	----------	----------	----------	----------	----------	----------	20 mm
Aeromonas scubertii	4 mm	----------	----------	12 mm	----------	----------	----------	15 mm	----------
Aeromonas hydrophila	6 mm	----------	----------	10 mm	----------	----------	----------	----------	22 mm
Aeromonas trota	6 mm	----------	----------	12 mm	----------	----------	----------	----------	----------
Aeromonas sobria	7 mm	----------	----------	9 mm	----------	----------	10 mm	----------	----------

R = Resistant (≤ 14mm) & I = Intermediate (15-18 mm) & S = Sensitive (≥ 19 mm)

Table 8. *Incidence of the Different Isolated Aeromonas species Sensitivity Against Oxytetracycline Disc (30 µg).*

Isolated strain		Group I (n=60)				Group II (n=60)				Group III (n=60)				Total
		R	I	S	Total	R	I	S	Total	R	I	S	Total	
A. caviae	**No.**	48	-	-	48	20	-	-	20	16	-	12	28	96
	%	34.29	-	-	34.29	31.25	-	-	31.25	80	-	60	50	36.92
A. scubertii	**No.**	32	-	-	32	16	-	-	16	-	16	-	16	64
	%	22.86	-	-	22.86	25.00	-	-	25.00	-	100	-	28.57	24.62
A. hydrophila	**No.**	24	-	-	24	8	-	-	8	-	-	8	8	40
	%	17.14	-	-	17.14	12.50	-	-	12.50	-	-	40	14.29	15.38
A. trota	**No.**	24	-	-	24	16	-	-	16	-	-	-	-	40
	%	17.14	-	-	17.14	25.00	-	-	25.00	-	-	-	-	15.38
A. sobria	**No.**	12	-	-	12	4	-	-	4	4	-	-	4	20
	%	8.57	-	-	8.57	6.25	-	-	6.25	20	-	-	7.14	7.69
Total	**No.**	140	-	-	140	64	-	-	64	20	16	20	56	260
	%	100	-	-	100	100	-	-	100	100	100	100	100	100

R = Resistant (≤ 14mm) & I = Intermediate (15-18 mm) & S = Sensitive (≥ 19 mm)

Table 9. *Interpretation Sensitivity of the Isolated Aeromonas species Against Oxytetracycline Disc (30 µg).*

Interpretation	Groups					
	I		II		III	
	No.	**%**	**No.**	**%**	**No.**	**%**
Resistant (R)	140	100	64	100	20	35.71
Intermediate (I)	-------	-------	-------	-------	16	28.57
Sensitive (S)	-------	-------	-------	-------	20	35.71
Total	140	100	64	100	56	100

Also the persistence of oxytetracycline at relatively high concentrations in the sediments for a significant period of time after medication exposed the tilapia for highly residual levels of oxytetracycline in their tissue [28].

The quantitative assessment of oxytetracycline residual levels in the examined tilapia samples were showed in table (4). The incidence of oxytetracycline detection in the examined samples of group I, II and III were 100% (60), 93.33% (60) and 53.33% (32) respectively. While the concentrations of oxytetracycline residues in the muscle of the tilapia were 319.92 ± 12.18, 142.14 ± 4.86 and 75.49 ± 1.66 ppb for group I, II and III respectively. The results of group I was agreed with the results recorded by Bernardy [29] while that of group II & III were lower. Also the obtained results were lower than that recorded by Choo [30]. The differences between the obtained results and results of other studies may be attributed to the variation in the administrated dose whereas the high dose leads to more rapid excretion than low [31]. It is result in an increase in the concentration of the antibiotic in the environment especially in the sediments [32, 28]. This increases the building up of antibiotic [33] and elongates antibacterial effects for 12 weeks within sediment after the cessation of treatment [27]. Also the variation in the time of samples obtaining, during or after the withdrawal time (21 day) may affect in the levels of antibiotic residues ([34, 13].

The abuse used of antibiotic either extensive uses of antibiotic to treat fish diseases nor added as feed additives in aquaculture could results of hazards of antibiotic residues, the antibiotic resistance bacteria and resistance (R) plasmid in fish and water microflora [35, 36]. Also the presence of antibiotic in sewage and treated water and the administration of animal manure as feed could increase the potential risk [34]; consequently produce fish constitute potential risks to the consumers [37, 38]. Such as increase the incidence of foodborne infections due to their association of antibiotic resistance enteric bacteria and treatment failure in human [20].

European Commission Council Regulation [39] set a standard permissible limit of oxytetracycline residual level in fish for human consumption. According to this limit, 100% (60) and 80% (48) of the group I and II were higher than the permissible limits and unfit for human consumption, while 13.33% (8) and 53.33% (32) of the group II and III were under the permissible limits and fit for human consumption. The incidence of the examined samples under the detection levels (<50ppb) of the HPLC were recorded in group II and III with a percentage of 6.67% (4) and 46.67% (28) respectively and considered fit for human consumption (table, 5). The mean values of the oxytetracycline levels were significant difference ($P<0.01$) between group I and II and in between I and III (table, 6). The variation in antibiotics levels between the three groups may be attributed to the variation in the factors affecting the levels of the antibiotic residues.

It could be isolated the strains of *A. caviae, A. scubertii, A. hydrophila, A. trota* and *A. sobria* from group I and II, with an incidence of 48 (34.29%), 32 (22.86%), 24 (17.14%), 24 (17.14%) and 12 (8.57%) and 20 (31.25%), 16 (25.00%), 8 (12.50%), 16 (25.00%) and 4 (6.25%) respectively (table, 7, 8, 9). For group III, it could be isolated strains of *A. caviae, A. scubertii, A. hydrophila* and *A. sobria* with an incidence of 7 (50%), 4 (28.57%), 2 (14.29%) and 4 (7.14%) respectively. Totally, the incidence of the isolated strain for group I, II and III were 140, 64 and 56 respectively. The highest incidences of isolates were *A. caviae* in the three groups. These results were higher than that recoded by Evangelista-Barreto [40] but lower than that reported by DePaola [41], Schmidt [42] and Schmidt

[43]. The variation in the number and types of the *Aeromonas* isolates from that of other studies may be attributed to the changes of water temperature and the adverse conditions during intensification [44], such as bad handling, inadequate concentration of feed and oxygen, salinity of the harvested site, concentration of organic matter and the sewage effluents of water [45, 5].

It was revealed that 100% of the isolated strains in group I (n=140) and II (n=64) were resistances for oxytetracycline, meanwhile 35.71% (n=20) of the isolated strains from the group III were resistances for oxytetracycline. The resistant strains of group III were identified as *A. caviae* and *A. sobria* that represent with an incidence of 80 % (n=16) and 20 % (n=4) respectively (table, 8, 9). The intermediates sensitive strains were recorded in group III and identifies as *A. scubertii* with an incidence of 100% (n=16) but the incidence of sensitive strains for oxytetracycline disc were recorded also in group III with an incidence of 60% (n=12) and 40% (n=8) for *A. caviae* and *A. hydrophila* respectively. The results of groups I and II agreed with the results recorded by Rhodes [46] and higher than that of Son [47], Schmidt [43] and Abraham[20]. The results of group III were lower than that recorded by Schmidt [42], Schmidt [43] and Abraham, [20]. These differences may due to the maintenance of the acquired resistance genes within the bacterial population, once it enter to these population, results in protection of the bacterium from tetracycline produced by other member of microflora or residues in agriculture or domestic effluents [43]. Also the misused of antibiotic such as of lower efficacy, high or lower dose of antibiotic [35, 36], the persistence and degradation of the antibiotic in the sediment, in the deeper layer, [48, 49] and an increase in the building up of antibiotic [50] enhance the production of bacterial resistance for antibiotic.

4. Conclusions

Antibiotics residues are seemed to quality defects in farmed tilapia that facing the challenges of the fish exporting in Egypt. Also, it is represent a potential health risks for consumers from the point of the adverse oxytetracycline residual effect and the initiation of the bacterial resistance genes. Application of good manufacture practices and Hazards Analysis Critical Control System (HACCP) in tilapia farms must be done for improving the antibiotics residual problem in fish muscles. Good management program should be applied to overcome the outbreak by *Aeromonas* species specially during the summer season. Treated tilapia must be held for the recommended withdrawal time before they can be marketed for ensuring their safety for human consumption. Future investigations should be done for evaluation the antibiotics residual levels in the water used for farming.

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STUDY ON TOKAJ WINE CONTAMINATION WITH DIFFERENT TYPE OF MOULDS

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ABSTRACT

Wine is and it will be one of the most consumed alcoholic drinks, both for its unique taste and its health benefits. Throughout time scientist had tried to stop as much as possible wine contamination, especially microorganism from the corks. Moulds are those that develop into the greatest extend and may be dangerous to humans, causing allergic reactions or respiratory problems.This paper shows the effect that different types of mold have on the Tokaj white wines, in terms of composition characteristics.

Keywords*: fungi, wine, contamination, Penicillium expansum, microscope picture, magnetic stirrer.*

1. Introduction

Even nowadays technology is advancing at lightning speed, microorganism wine contamination is not excluded. One of the actual problems that wine industry faces is wine contamination with microorganism from the corks. The purpose of this paper is to follow the Tokaj white wine contamination with three different types of fungi. The types of wine are: Dry Furmint, Aszú 4 putony, Sweet Szamorodni [1, 2].

Fungi were isolated from samples obtained from contaminated cork (corkwood) stoppers. Three types of isolated fungi were used: *Penicillium chrysogenum, Penicillium expansum, Phanerochaete chrysosporium* (Figures 1, 2, 3) [3].

The identification of the above fungi were made on the basis of their macroscopic (cultural and morphological characters on agar medium) and microscopic structures: the formed of individual conidia the chains of conidia, the structure and types of conidiophores.

2. Materials and methods

2.1 Materials

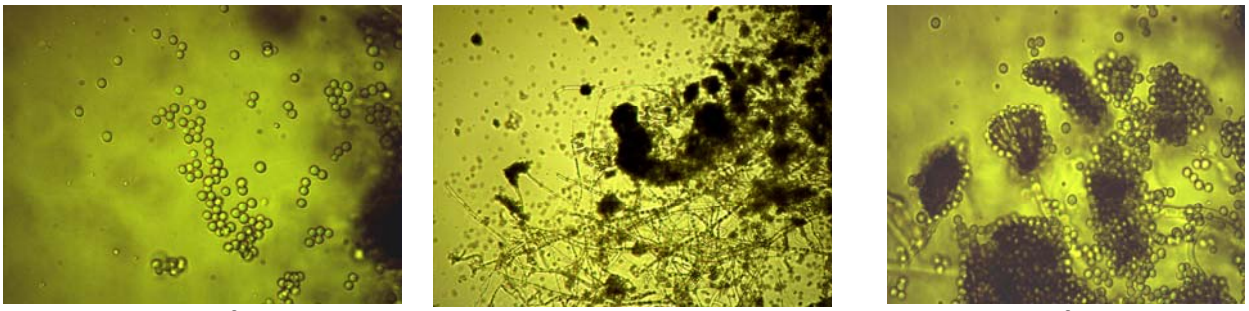
Three types of wine was used for experiment development: (i) Dry Furmint; (ii) Sweet Szamorodni and (iii) Aszu 4 putony.

2.2 Methods

Each type of wine was divided in four Erlenmayes glasses, obraining twelve samples. Three samples were preserved intact and the remaining samples were inoculated with the three types of mold. Then the glasses were agitated for three weeks on the magnetic stirrer (Figures 4, 5).

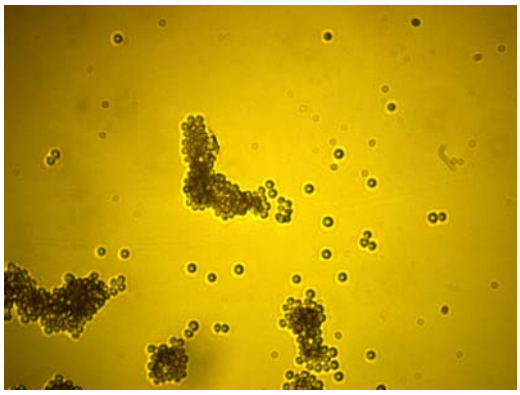
The goal was to obtain from each type of wine a healthy sample, the second with *Penicillium chrysogenum*, the third with *Penicillium expansium* and the fourth with *Phanerochaete chrysosporium*.

Microorganisms were isolated from damaged cork samples, plated fragments into PDA plates (potatoes dextrose agar).



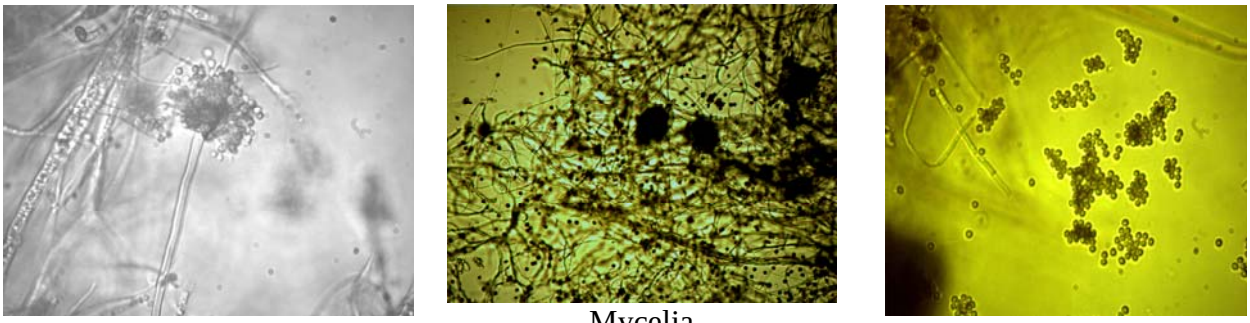
Conidia form | Mycelia | Chain of conidia

Figure 1. Penicillium chrysogenum microscope pictures



Conidia form

Figure 2. Penicillium expansum microscope pictures



Conidiophore | Mycelia | Hyphal chains

Figure 3. Phanerochaete chrysosporium microscope pictures

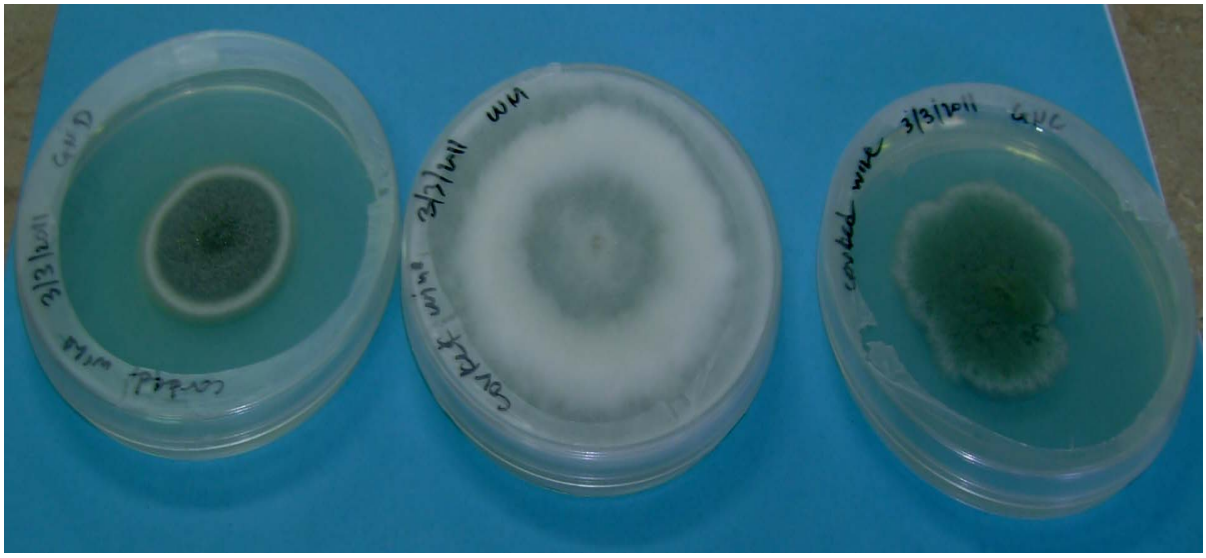


Figure 4. Fungi cultures

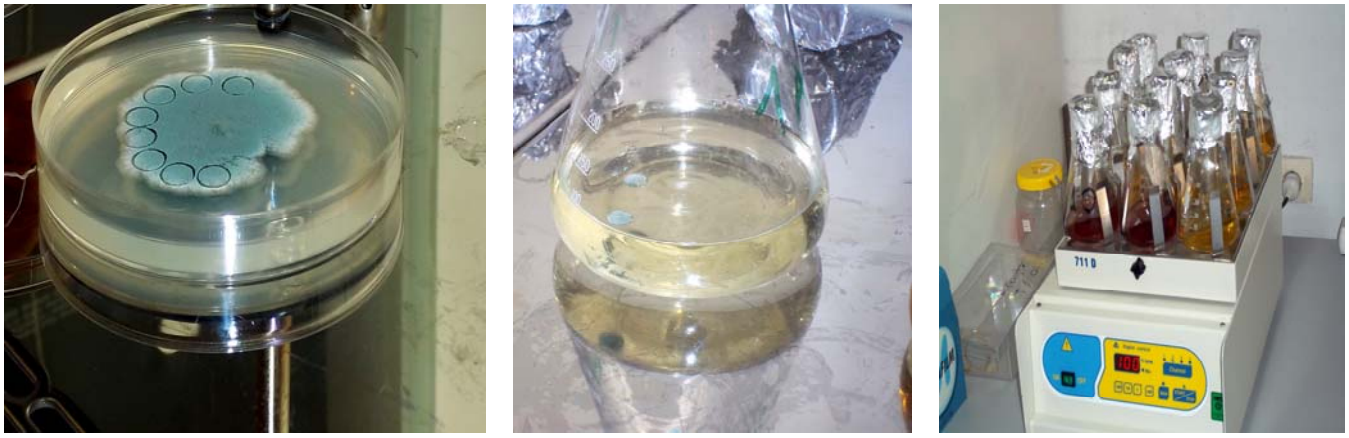


Figure 5. Wine contamination process

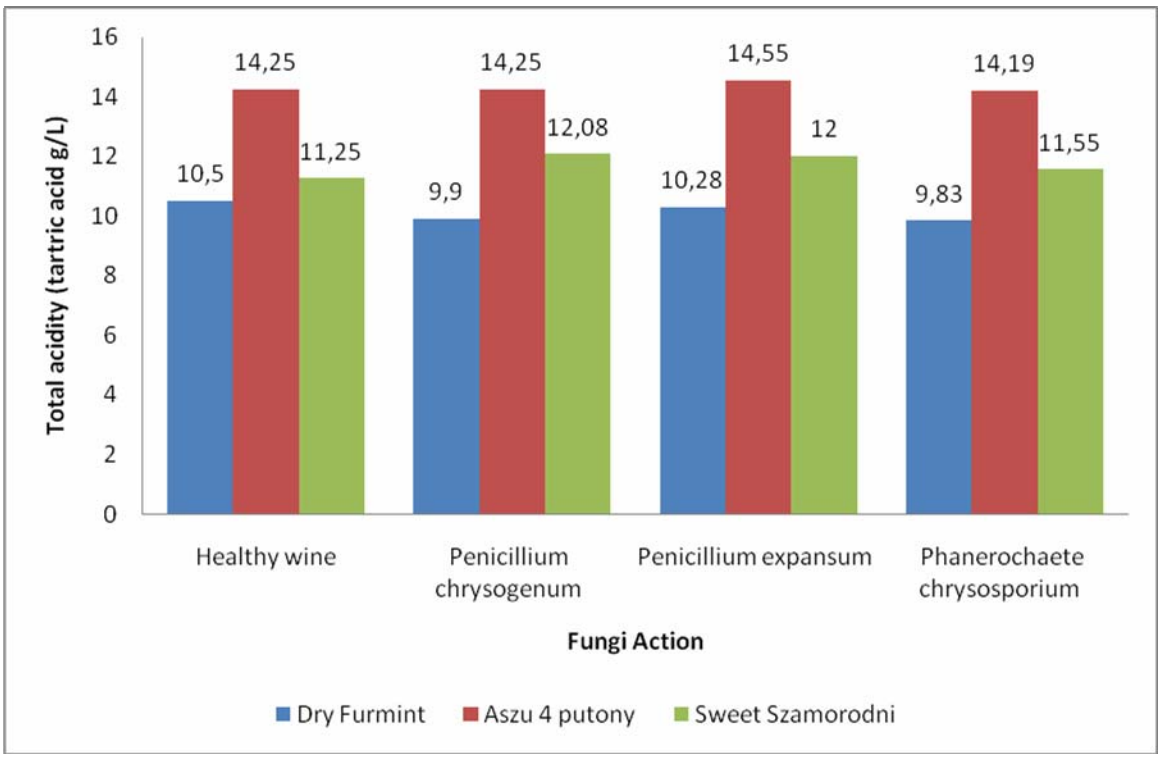


Figure 6. Variation of total acidity on the three different types of wine

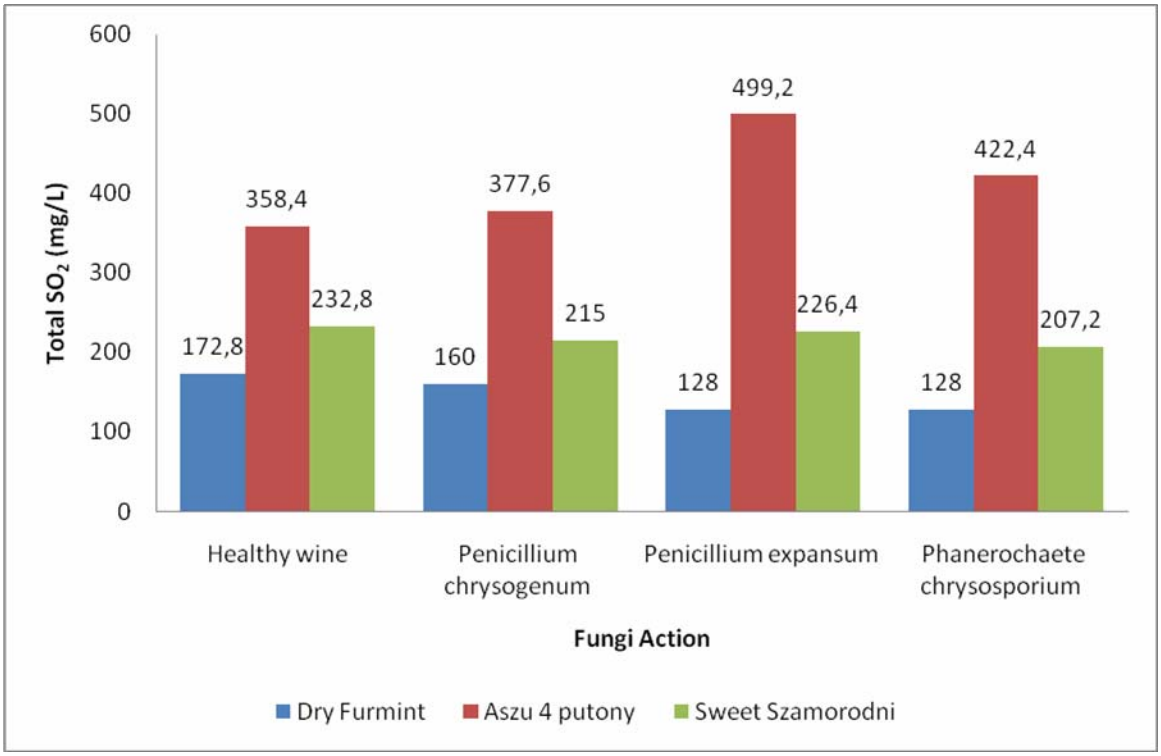


Figure 7. Variation of total SO_2 on the three different types of wine

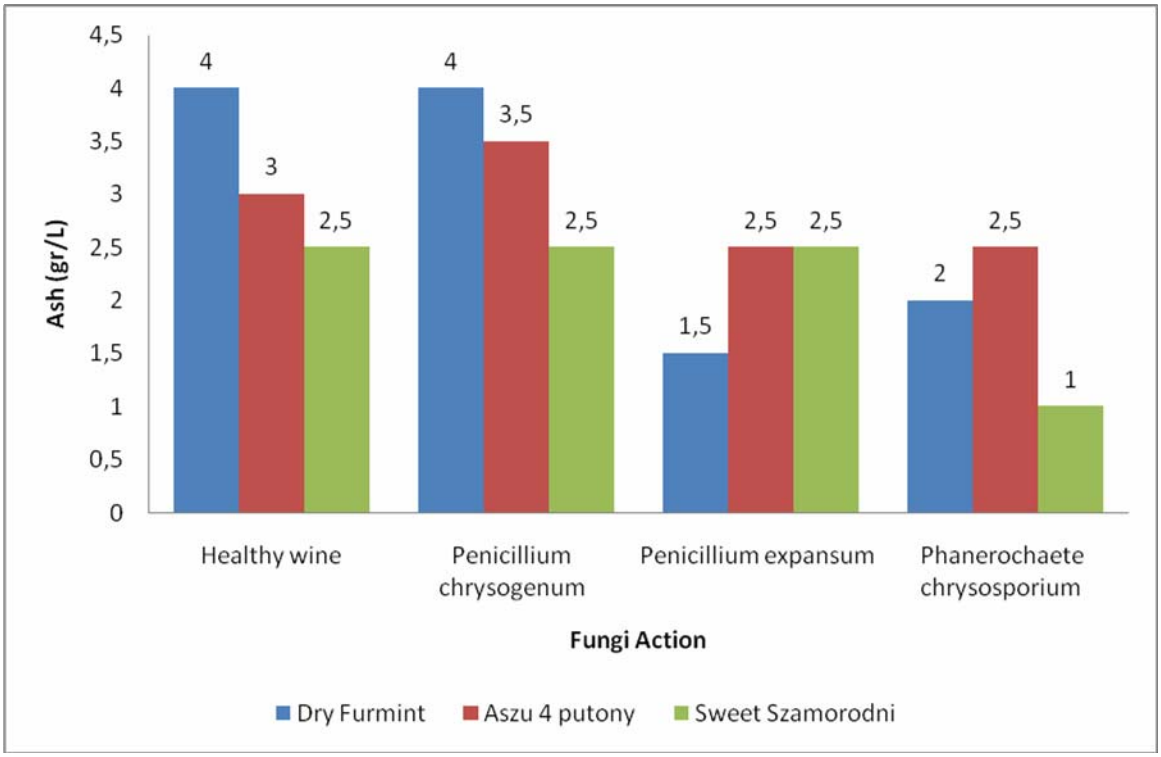


Figure 8. Variation of ash on the three different types of wine

After 4 days of incubation at 25^0C, individual fungal colonies were sub-cultured under the same condition, and pure cultures were obtained. Microscopic identification of each the type of fungi was performed.

The next analysis were conduct for wine samples:

a) Total Acidity

The method consist in neutralizing the acidity with solution of NaOH 0.1 N, using bromothymol as an indicator. Acidity was expressed as tartric acid g/L [4, 5, 6].

b) Total SO2

The method consists in titration the sample to be analyzed with solutions of NaOH and H_2SO_4 and the iodomoetric titration of the released sulfure dioxid from its combination with sugar and aldehydes in acidic medium. The results were expressed as SO_2 mg / L [4, 5, 6].

c) Ash

The method consists in evaporating the wine to be analyzed on water bath and calcination the dried residue in an electric furnace at temperature range of 450-550^{0}C. [4, 5, 6].

3. Results and discussions

Three types of fungus were identified on the 12 analyzed wine samples: *Penicillium chrysogenum*, *Penicillium expansum* and *Phanerochaete chrysosporium.*

According to Figures 6 - 8 the healthy wine is within the normal values. The color of the wines did not changed at all.

The Figure 6 indicates the variation of total acidity different for each type of fungi. *Penicillium chrysogenum* lowers the value of acidity for each type of wine comparing to healthy wine, so this means that the wine was not contaminated, the wine is not acidulated. *Penicillium expansum* and *Phanerochaete chrysosporium* increase the

value of acidity, the wine is contaminated, not good for consumers and the taste is acidulated.

Regarding the quantity of total SO_2 (Figure 7) things change. *Penicillium chrysogenum* and *Phanerochaete chrysosporium* lowered the amount of total SO_2, while *Penicillium expansum* raises the values.

The amount of ash for healthy wine should be framed in concentration range 1.3 to 4 g/l. According to the Figure 8 the quantity of ash for all types of wines is maximum 4 g/L. So the wines were not damaged.

4. Conclusions

Based on above experiments, we can conclude that not every type of fungus can damaged and contaminate the wine. In the same time, even if the three fungi had changes some values of the wine composition, especially the taste, acidity and total sulf dioxide, this doesn't mean that the wine is fully damaged, but the consumers are not allowed to drink it because it may cause health problems

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OIL YIELDS, FATTY ACID COMPOSITIONS AND TOCOPHEROL CONTENTS OF GRAPE SEED OILS FROM TURKEY

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ABSTRACT

In the present study, the oil contents, fatty acid composition and tocopherol contents of grape seed and corresponding oils were analyzed by using Gas Chromatograph and High Performance Liquid Chomotography. The results showed that the oil concentration of seeds ranged from 7.9 to 20.1 % Grape seed oils were rich in oleic and linoleic acids, ranging from 12.9 to 27.1 % and 58.3 to 74.8 %, respectively. In addition, A few types of tocopherols were found in grape seed oils in various amount: α-tocopherol, α-tocotrienol, γ-tocopherol, β-tocotrienol and α-tocotrienol. As a result, present study shows that oil, fatty acid composition and tocopherol contents differ significantly among the cultivars.

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Keywords*:* grape seed oil, fatty acid composition, gas chromatograph, tocopherol.

1. Introduction

The potential and current sources of oils, not only restricted to seeds, but also are included in food industry by-products. Grape seeds are waste products of the wine and grape juice industry [1]. Grape seed oil is generating increased interest as a functional food product since it has been shown to contain high levels of vitamin E and unsaturated fatty acids. It is well known that oils obtained from grape by-products can be used for pharmaceutical and nutritional purposes [2]. These by-products contain some valuable substances such as fatty acids, tocopherols, sterols and sterylglycosides with potential applications in food industry mostly go to waste. On the other side, grape pressing in wineries resulted in huge amounts of grape pomace containing grape seeds that are used as a source of oil having nutritional and pharmaceutical implications [1, 2, 3, 4, 5, 6]. Tocopherols are regarded as intracellular antioxidants due to their activity in inhibiting the peroxidation of polyunsaturated fatty acids in biological membranes. Most of the commonly used vegetable oils contain only tocopherols. Commercial grape seed oil contains a relatively high quantity of the vitamin E isomers, particularly α-and γ-T3 up to about 35.2 and 78.5 mg/100g oil, respectively [7, 5, 6] The source of oils and fats is diminishing, this means that there is the growing need for the search of new sources of oil as well as exploiting sources that are currently unexploited in order to supplement the existing ones [8].

The aim of this study was to determine oil yield, fatty acid composition and tocopherol contents of oil extracted from different grape seeds collected several locations of Turkey.

2. Materials and methods

2.1.Material

The ripened grape fruits were collected from different locations and provinces of Turkey, and were separately

crushed by hand, and their pulps were removed from seeds. The seeds were washed with water and left to air-dry for 3 days. Then, the seeds were dried in atmospheric conditions. The seeds were comminuted into pieces including a previously cleaned. The dried materials were finely ground in a mortar, and the seed pieces were then stored in an air-tight container in a refrigerator (-20°C) prior to analysis.

2.2. Reagents

Petroleum ether (40-60^{o}C) was of analytical grade (>98%; Merck, Darmstadt, Germany). Heptane and tert-butyl methyl ether were of HPLC grade (Merck, Darmstadt, Germany). Tocopherol and tocotrienol standard compounds were purchased from CalBiochem (Darmstadt, Germany).

2.3. Oil extraction

Grape seed oil was obtained by extraction of the meal with petroleum ether (50^{o}C) in a Soxhlet extractor for 6 h. After extraction of the oil, the solvent was evaporated under reduced pressure. The obtained oil was kept in sealed glass bottles under deep freezing (-18^{o}C) for further analysis.

2.4. Fatty Acid Composition

The fatty acid composition was determined following the ISO standard ISO 5509:2000 (ISO 2000). In brief, one drop of the oil was dissolved in 1 mL of n-heptane, 50 μg of sodium methylate was added, and the closed tube was agitated vigorously for 1 min at room temperature. After addition of 100 μL of water, the tube was centrifuged at 4500 g for 10 min and the lower aqueous phase was removed. Then 50 μL of HCl (1 mol with methyl orange) was added, the solution was shortly mixed, and the lower aqueous phase was rejected. About 20 mg of sodium hydrogen sulphate (monohydrate, extra pure; Merck, Darmstadt, Germany) was added, and after centrifugation at 4500 g for 10 min, the top n-heptane phase was transferred to a vial and injected in a Varian 5890 gas chromotograph with a capillary column, CP-Sil 88 (100 m long, 0.25 mm ID, film thickness 0.2 μm). The temperature program was as follows: from 155 °C; heated to 220 °C (1.5^{o}C/min), 10 min isotherm; injector 250°C, detector 250°C; carrier gas 36 cm/s hydrogen; split ratio 1:50; detector gas 30 mL/min hydrogen; 300 mL/min air and 30 mL/min nitrogen; manual injection volume less than 1 μL. The peak areas were computed by the integration software, and percentages of fatty acid methyl esters (FAME) were obtained as weight percent by direct internal normalization.

2.5. Tocopherols

For determination of tocopherols, a solution of 250 mg of oil in 25 mL of n-heptane was directly used for the HPLC. The HPLC analysis was conducted using a Merck-Hitachi low-pressure gradient system, fitted with a L-6000 pump, a Merck-Hitachi F-1000 fluorescence spectrophotometer (detector wavelengths for excitation 295 nm, for emission 330 nm), and a D-2500 integration system. The samples in the amount of 20 μL were injected by a Merck 655-A40 autosampler on to a Diol phase HPLC column 25 cm x 4.6 mmID (Merck, Darmstadt, Germany) used with a flow rate of 1.3 mL/min. The mobile phase used was n-heptane/tert-butyl methyl ether (99+1, v/v [9].

Each method was carried out in triplicate for each sample. The mean values were given in the tables, without the standard deviation, because this value would represent only the deviation of the method and not the variation of the appropriate sample.

3. Results and discussions

3.1. Oil contents

The oil contents of grape seeds are given in Table 1. The oil contents of materials ranged between 7.9 % to 20.1 %. While the highest oil content is found in Su grape seed, the oil was established in İrikara grape seed. The crude oil contents of grape seeds were similar to those for grape seeds reported by Göktürk Baydar and Akkurt [4], Göktürk Baydar et al. [5], Ohnishi et al. [3], Schester [10] and Özcan et al. [11]. Hassanein and Abadel-Razek [2] established 12.0 % oil in grape seed. Göktürk Baydar et al. [5] reported that the oil concentration of grape seeds ranged from 12.35 % to 16.00 %. In other study, the oil level of grape seeds were found between 11.6 % to 19.6 % [3].

Table 1. *Oil contents of grape seeds (%)*

Grape names	*Oil contents, %*
At püskülü	16.4
Kozak beyazı	13.9
Söbe kara	15.6
Barinak	15.6
Dimrit siyah	17.3
Antep karasi	3.7
Isbitiren Konya	11.2
Adana beyazı	11.7
Misket	14.2
Büzgülü Gülnar	16.0
Kizil Bozkır	9.6
Göküzüm	13.0
Hönüsü siyah Antep	17.6
Kozak siyahi	16.7
Kadin parmagi beyaz	12.9
Müsgüle beyaz	18.1
Tarsus beyazi mersin	16.2
Hesapali Konya	19.7
Dökülgen Konya	12.1
Büzgülü siyah Isparta	11.6
Aküsüm Doganhisar	14.1
Gök üzüm Konya	15.3
Irikara	7.9
Tarsus beyazi Gülnar	14.3
Dimrit siyah Doganhisar	7.2
Miski Gülnar	16.0
Muftalma	13.5
Devegözü Doganhisar	16.0
Incekabuk Bozkir	14.6
Kardinal Büyükeceli	9.1
Aküzüm Konya	17.4
Büzgülü Konya	14.0
Topacik beyaz Beysehir	11.7
Recep Büyükeceli	18.2
Marcas Gülnar	13.5
Dimlit Taskent	16.3
Nazli beyaz Konya	15.7
Koz Kargicak	14.8
Hönüsü siyah Büyükeceli	17.4
Cavus beyaz Konya	18.4
Kizil üzüm Doganhisar	12.5
Isbitiren Taskent	15.0
Takara siyah Gülnar	18.1
Su üzümü Konya	20.1
Sergi Gülnar	17.7
Redglob murtici	16.6
Razaki beyaz Antep	16.6
Eksi kara Hadim	13.3
Toros beyazi Büyükeceli	17.7
Topacik siyah Konya	12.6
Karadimlit Taskent	19.9
Beylerce Denizli	12.5
Alyanak Denizli	14.7
Bogazkere Denizli	16.1
Alfons Denizli	13.1
Öküzgözü Denizli	13.9
Izazdag Denizli	12.6
Dimlit Denizli	11.7
Retglob Denizli	17.8
Soltaniye Denizli	8.6

3.2. Fatty acid contents

The fatty acid compositions of the grape seed oils extracted from sixty different grape seeds are presented in Table 2. The fatty acid composition of seed oil triacylglycerides varies widely among different plant species and often the occurrence of unusual fatty acids is characteristic for particular plant families [11]. According to the results shows in Table 3, the most predominant fatty acid of all seed

oils of grape was lineloic acid, which accounted for 58.3 % (Çavuşbeyazı) to 74.8% (Dimlit) in oils. In addition linoleic acid, seed oils grape contained higher amounts of oleic acid. The range of oleic acid was between 12.9 % (Irikara) to 27.1 % (Razaki beyaz). The seed oils of grape also contained appreciable amounts of saturated fatty acids, especially palmitic and stearic acids. In previous study, the fatty acid contents of the grape seed oils had the following range: 7.42 to 10.24 % for palmitic, 2.95 to 4.68 for stearic, 16.15 to 21.63 % for oleic, 63.33 to 71.37 % for linoleic and 0.14 to 0.35 % for linolenic acid [5]. Grape seeds were rich in oleic and linoleic acids, ranging from 17.8 to 26.5 % and 60.1 to 70.1 %, respectively [4]. Uslu and Dardeniz [12] reported that grape seed cultivars contained 8.40-6.51 % palmitic, 16.10-11.62 % oleic, 77.59-72.50 % linoleic, 3.86-3.07 % stearic acids, showing that grape oil had the quality of a good cooking oil. Özcan et al. [11] determined 4.1 % palmitic, 10.4 % stearic, 16.4 % oleic and 69.3 % linoleic acids in grape seed oil. Grape seed oil was rather poor in linolenic acid. Low levels of linolenic acid are desired in edible oils, because high levels of this fatty acid can produce an unfavourable odour and taste in oil [13, 14, 5].

The range of concentrations of the fatty acids was similar to the previous published data. The variations observed between the results of this work could be probably due to differences in climatic conditions, soil structure, localities and environmental temperature during maturation of grape seeds.

3.3. Tocopherol contents

α-T, α-T3, β-T, γ-T, β-T3, p8, γ-T3, б-T, б-T3 and total tocopherol contents of oil extracted from several grape cultivars are shown in Table 3. Generally, α-Tocopherol, α-tocotrienol and γ-tocotrienol were established at the high levels. While β-tocotrienol was not detected in the oil grape seeds (except for Toros beyazı, Topacık siyah, Beylerce Öküzgözü and Retglob grapes), γ -tocotrierol, α-tocotrierol and α-tocopherol were the most abundant tocopherol in the grape seed oils. In addition, tocopherol contents ranged from 0.8 to 26.4 for α-tocopherol, 1.4 to 36.8 for α-tocotrienol, 0.2 to 6.5 for γ -tocopherol and 5.7 to 62.6 for γ-tocotrienol in the seed oil. The seed oils of Devegözü, Beylerce, Alyanak and Öküzgözü exhibited the highest α-tocopherol values as compared to the other cultivars. On the other hand, α-tocotrierol contents of grape seed oils were found between 1.4 (Büzgülü siyah) to 36.8 (Razaki beyaz). Also, γ -tocotrienol contents ranged between 5.7 (Büzgülü siyah) to 62.6 (Kardinal). In addition, total tocopherol contents were determined between 7.8 (Büzgülü siyah) to 103.2 (Beylerce). The highest total tocopherol contents of seed oils were determined in Devegözü (89.9), Kardinal (88.1), Redglob (90.5) and Beykerce (103.2). Wie et al [6] reported that the total concentration of tocopherol and tocotrienol was in the range of 4.8-9.9 mg/ 100 g seed (35.3-68.8 mg/ 100g oil basis). The Muscat Bailey A cultivar had the highest total tocopherol and tocotrienol contents, followed by Canner and Naples. γ-tocotrienol ranged from 1.6 to 4.9 mg/100 g seed (11.2 to 53.81 mg/100 g oil basis) and was the main isomer, followed by α-tocotrienol in most of the samples [6].

Göktürk Baydar and Akkurt [4] reported that total tocopherol in grape seed oil from 18 different cultivars ranged from 32.8 to 57.8 mg/100g oil. The levels of tocopherols and tocotrienols in grape seeds are comparable with palm and bran oils based on oil content. Tocotrienols have unique physiological activities, including hypo cholesterolemic, anti-thrombotic, antiproliferative, and neuroprotective properties [15]. Grape seed oil contained 10.0 % α-tocopherol, 2.5 % γ -tocopherol, 37.5 % α-tocotrienol and 50.0

% γ-tocotrienol [2]. Göktürk Baydar et al. [5] determined 128.14-325,39 mg/kg α-tocopherol, 14.37-39.31 mg/kg γ-tocopherol 0.62-1.63 mg/kg б-tocopherol in different grape seed oils. Differences in tocopherol contents of grape seed oil are depend on genotype [4, 16].

Table 2. *Fatty acid compositions of grape seed oils (%)*

Grape name	16:0	16:1n-7	16:1n-9	18:0	18:1n-9	18:1n-7	18:2n-6	20:1n-9	18:3D9,12,15	20:1n-7	18:4D6,9,12,15	Total (%)
At püskülü	8.2	0.1	0.1	4.1	16.9	0.8	68.6	0.2	0.4	0.0	0.2	99.7
Kozak beyazi	9.1	0.2	0.1	4.7	19.8	0.2	65.2	0.2	0.0	0.4	0.2	99.9
Söbe kara	8.7	0.1	0.1	3.6	17.1	0.2	68.1	0.2	0.0	0.5	0.2	98.6
Barinak	8.2	0.2	0.1	4.3	26.4	0.3	59.4	0.2	0.0	0.3	0.2	99.5
Dimrit siyah	8.0	0.1	0.1	5.1	18.9	0.7	65.7	0.2	0.0	0.4	0.2	99.5
Antep karasi	12.1	0.3	0.3	4.8	19.6	1.2	59.2	0.2	0.6	0.0	0.2	98.5
Isbitiren Konya	7.6	0.1	0.0	4.2	17.1	0.8	69.5	0.2	0.3	0.0	0.2	99.9
Adana beyazi	8.4	0.3	0.0	5.5	17.3	0.9	66.5	0.2	0.0	0.4	0.2	99.7
Misket	7.1	0.1	0.0	4.1	17.3	0.7	69.7	0.2	0.0	0.5	0.2	99.8
Büzgülü Gülnar	8.2	0.1	0.0	3.7	16.2	0.8	70.1	0.2	0.4	0.0	0.2	99.8
Kizil Bozkir	8.3	0.1	0.0	4.7	16.4	0.7	68.8	0.2	0.4	0.0	0.2	99.8
Göküzüm	7.9	0.1	0.0	4.2	17.5	0.8	68.7	0.2	0.3	0.0	0.2	99.8
Hönüsü siyah Antep	9.6	0.2	0.0	4.3	21.8	1.1	61.8	0.2	0.4	0.0	0.2	99.4
Kozak siyahi	7.6	0.1	0.0	4.2	15.1	0.8	70.8	0.1	0.4	0.0	0.2	99.4
Kadin parmagi beyaz	8.5	0.2	0.0	5.1	20.1	0.0	65.2	0.2	0.3	0.0	0.1	99.5
Müsgüle beyaz	6.7	0.0	0.1	5.5	16.5	0.8	69.1	0.0	0.2	0.3	0.0	99.1
Tarsus beyazi mersin	7.9	0.1	0.0	5.5	18.7	0.8	66.6	0.0	0.3	0.0	0.0	100.0
Hesapali Konya	8.5	0.1	0.0	6.3	22.5	0.9	60.8	0.0	0.3	0.0	0.0	99.4
Dökülgen Konya	8.0	0.0	0.0	4.3	16.8	0.8	68.9	0.0	0.0	0.0	0.0	98.8
Büzgülü siyah Isparta	8.2	0.3	0.0	5.0	17.5	1.1	65.6	0.0	0.4	0.0	0.0	98.0
Aküsüm Doganhisar	7.2	0.1	0.0	4.5	14.2	1.0	71.5	0.0	0.4	0.0	0.2	99.1
Gök üzüm Konya	8.4	0.0	0.0	4.0	18.4	0.0	68.4	0.0	0.4	0.0	0.0	99.6
Irikara	8.3	0.1	0.0	3.5	12.9	1.3	71.2	0.2	0.0	0.0	0.0	97.6
Tarsus beyazi Gülnar	8.6	0.2	0.0	4.7	18.4	1.0	65.6	0.2	0.4	0.0	0.2	99.4
Dimrit siyah Doganhisar	7.9	0.1	0.0	4.5	13.5	0.8	72.2	0.2	0.5	0.0	0.2	99.9
Miski Gülnar	7.2	0.1	0.0	4.2	14.2	0.6	72.8	0.1	0.3	0.0	0.2	99.9
Muftalma	6.9	0.1	0.0	4.9	21.5	0.7	64.6	0.2	0.0	0.4	0.2	99.5

Table 2.

Grape name	16:0	16:1n-7	16:1n-9	18:0	18:1n-9	18:1n-7	18:2n-6	20:1n-9	18:3D9,12,15	20:1n-7	18:4D6,9,12,15	Total (%)
Devegözü Doganhisar	9.2	0.1	0.0	3.9	16.0	0.8	68.5	0.0	0.4	0.2	0.0	99.2
Incekabuk Bozkir	7.8	0.1	0.0	4.5	21.6	1.1	64.1	0.2	0.3	0.0	0.2	99.9
Kardinal Büyükeceli	8.3	0.2	0.0	3.9	15.8	0.9	68.1	0.2	0.4	0.0	0.2	97.9
Aküzüm Konya	7.8	0.1	0.0	6.4	22.4	0.8	61.4	0.2	0.3	0.0	0.2	99.7
Büzgülü Konya	7.5	0.1	0.0	4.9	15.2	0.8	70.5	0.2	0.3	0.0	0.2	99.6
Topacik beyaz Beysehir	7.4	0.1	0.0	5.1	15.4	0.7	69.9	0.2	0.4	0.0	0.2	99.5
Recep Büyükeceli	9.5	0.2	0.0	4.5	18.0	1.0	66.1	0.1	0.3	0.0	0.1	99.7
Marcas Gülnar	8.3	0.1	0.0	4.6	17.0	0.8	67.5	0.0	0.4	0.0	0.2	98.9
Dimlit Taskent	7.4	0.2	0.0	4.1	16.5	0.8	69.9	0.0	0.4	0.2	0.0	99.5
Nazli beyaz Konya	7.3	0.2	0.0	4.3	16.6	0.8	69.7	0.2	0.3	0.0	0.2	99.5
Koz Kargicak	9.6	0.2	0.0	6.2	19.7	0.8	62.6	0.2	0.4	0.0	0.2	99.9
Hönüsü siyah Büyükeceli	8.0	0.1	0.0	4.7	20.4	0.8	64.9	0.0	0.3	0.2	0.0	99.5
Cavus beyaz Konya	9.1	0.2	0.0	5.6	24.8	1.1	58.3	0.0	0.3	0.2	0.0	99.6
Kizil üzüm Doganhisar	8.2	0.1	0.0	4.8	16.6	0.9	68.1	0.0	0.4	0.2	0.0	99.3
Isbitiren Taskent	7.4	0.1	0.0	4.5	17.0	0.7	69.2	0.2	0.3	0.0	0.2	99.6
Takara siyah Gülnar	7.4	0.1	0.0	4.5	14.4	0.7	71.2	0.2	0.3	0.0	0.2	99.2
Su üzümü Konya	7.7	0.1	0.0	3.6	15.8	0.7	71.1	0.2	0.3	0.0	0.2	99.7
Sergi Gülnar	7.8	0.1	0.0	6.0	17.4	0.7	67.0	0.2	0.3	0.0	0.2	99.7
Redglob murtici	7.7	0.1	0.0	4.7	18.2	0.8	67.5	0.2	0.3	0.0	0.2	99.7
Razaki beyaz Antep	8.5	0.1	0.0	4.7	27.1	1.0	57.5	0.2	0.3	0.0	0.2	99.7
Eksi kara Hadim	7.6	0.1	0.0	5.1	13.8	0.8	71.4	0.2	0.3	0.0	0.2	99.5
Toros beyazi Büyükeceli	8.3	0.1	0.0	5.2	20.8	0.8	63.9	0.2	0.3	0.0	0.2	99.8
Topacik siyah Konya	8.0	0.1	0.0	4.0	17.5	0.8	68.4	0.0	0.4	0.0	0.2	99.4
Karadimlit Taskent	7.9	0.1	0.0	4.5	16.6	0.7	69.2	0.2	0.4	0.0	0.2	99.8
Beylerce Denizli	8.1	0.1	0.0	4.3	16.3	0.7	69.1	0.2	0.5	0.0	0.2	99.6
Alyanak Denizli	9.0	0.2	0.0	3.9	18.8	0.8	66.4	0.2	0.5	0.0	0.2	99.8
Bogazkere Denizli	7.0	0.1	0.0	5.5	22.6	0.7	63.4	0.2	0.4	0.0	0.2	99.9
Alfons Denizli	8.0	0.2	0.0	3.8	17.5	0.8	68.4	0.0	0.2	0.0	0.2	99.1
Öküzgözü Denizli	8.8	0.2	0.1	4.5	19.3	1.0	65.5	0.2	0.4	0.0	0.0	99.9
Izazdag Denizli	9.1	0.1	0.0	5.2	19.0	0.8	64.9	0.2	0.4	0.0	0.2	99.9
Dimlit Denizli	7.8	0.1	0.0	3.2	12.2	0.9	74.8	0.1	0.5	0.0	0.2	99.6
Retglob Denizli	7.7	0.1	0.0	4.2	16.6	0.8	70.0	0.0	0.3	0.0	0.2	99.9
Soltaniye Denizli	8.4	0.1	0.0	4.7	18.4	0.8	66.5	0.0	0.5	0.2	0.0	99.6

Table 3. *Tocopherol contents of grape seed oils (mg/100 g oil)*

Name	α-T	α-T3	β-T	γ-T	β-T3	P8	γ-T3	δ-T	δ-T3	Total
At püskülü	5.2	8.7	0.0	0.5	0.0	1.1	10.1	0.0	0.0	25.6
Kozak beyazi	2.1	11.0	0.0	0.6	0.0	0.8	5.7	0.0	0.0	20.3
Söbe kara	2.3	10.4	0.0	0.7	0.0	0.3	9.6	0.0	0.0	23.2
Barinak	3.6	20.5	0.0	2.0	0.0	0.5	8.5	0.0	0.0	35.0
Dimrit siyah	1.7	12.0	0.0	0.3	0.0	0.9	16.4	0.0	0.0	31.3
Antep karasi	3.2	35.3	0.0	1.8	0.0	1.0	8.1	0.0	0.0	49.5
Isbitiren Konya	3.2	9.7	0.0	0.9	0.0	0.0	19.9	0.0	0.0	33.7
Adana beyazi	7.1	21.8	0.0	1.3	0.0	0.0	31.4	0.0	0.0	61.6
Misket	7.6	9.2	0.0	1.5	0.0	1.0	26.8	0.0	0.0	46.2
Büzgülü Gülnar	6.8	15.3	0.0	0.7	0.0	0.0	25.5	0.0	0.0	48.3
Kizil Bozkir	7.6	12.5	0.0	3.1	0.0	1.3	23.4	0.0	0.0	47.9
Göküzüm	5.1	17.6	0.0	0.6	0.0	1.0	23.1	0.0	0.0	47.4
Hönüsü siyah Antep	10.2	23.8	0.0	3.0	0.0	1.4	20.5	0.0	0.0	58.8
Kozak siyahi	17.8	20.9	0.0	1.8	0.0	3.7	34.3	0.0	0.0	78.5
Kadin parmagi beyaz	7.6	22.5	0.0	0.0	0.0	1.8	16.6	0.0	0.0	48.5
Müsgüle beyaz	13.0	25.0	0.0	1.9	0.0	0.4	27.5	0.0	0.0	67.8
Tarsus beyazi mersin	13.4	16.2	0.0	2.9	0.0	2.4	26.6	0.0	0.0	61.6
Hesapali Konya	4.0	21.6	0.0	0.5	0.0	1.1	21.2	0.0	0.0	48.4
Dökülgen Konya	1.3	4.2	0.0	0.9	0.0	13.1	0.0	0.0	0.0	19.4
Büzgülü siyah Isparta	0.5	1.4	0.0	0.2	0.0	0.0	5.7	0.0	0.0	7.8
Aküsüm Doganhisar	2.0	2.6	0.0	1.6	0.0	0.0	9.9	0.0	0.0	16.1
Gök üzüm Konya	2.1	8.8	0.0	1.2	0.0	0.7	22.1	0.0	0.0	34.9
Irikara	0.8	2.7	0.0	1.7	0.0	0.0	11.2	0.0	0.0	16.4
Tarsus beyazi Gülnar	9.7	13.1	0.0	1.4	0.0	1.0	34.4	0.0	0.0	59.7
Dimrit siyah Doganhisar	7.7	7.4	0.0	2.6	0.0	0.9	20.9	0.0	0.0	39.5
Miski Gülnar	2.8	6.8	0.4	0.7	0.0	0.1	20.8	0.0	0.0	31.5
Muftalma	2.0	6.0	0.0	0.6	0.0	0.2	18.8	0.0	0.0	27.6
Devegözü Doganhisar	18.9	20.8	0.0	2.3	0.0	1.3	46.6	0.0	0.0	89.9
Incekabuk Bozkir	10.6	11.9	0.0	1.0	0.0	0.8	23.5	0.0	0.0	47.8
Kardinal Büyükeceli	5.8	18.1	0.0	1.1	0.0	0.6	62.6	0.0	0.0	88.1
Aküzüm Konya	5.0	23.6	0.0	1.0	0.0	1.1	20.1	0.0	0.0	50.8
Büzgülü Konya	1.2	2.9	0.0	0.7	0.0	0.0	26.8	0.0	0.0	31.7
Topacik beyaz Beysehir	8.8	24.2	0.0	3.4	0.0	1.2	36.7	0.0	0.0	74.3
Recep Büyükeceli	6.8	19.8	0.0	1.1	0.0	0.9	43.7	0.0	0.0	72.3
Marcas Gülnar	8.4	12.5	0.0	2.6	0.0	0.9	19.6	0.0	0.0	44.0
Dimlit Taskent	7.0	18.3	0.0	1.7	0.0	0.5	34.2	0.0	0.0	61.7
Nazli beyaz Konya	6.9	19.6	0.0	1.2	0.0	0.4	35.0	0.0	0.0	63.2
Koz Kargicak	12.6	31.1	0.0	0.2	0.0	2.2	15.0	0.0	0.0	61.1
Hönüsü siyah Büyükeceli	14.7	18.1	0.0	0.7	0.0	0.8	33.4	0.0	0.0	67.7
Cavus beyaz Konya	10.3	24.8	0.0	0.9	0.0	1.8	24.1	0.0	0.0	61.9
Kizil üzüm Doganhisar	6.7	11.6	0.0	1.3	0.0	0.0	30.1	0.0	0.0	49.7
Isbitiren Taskent	5.7	23.8	0.0	1.0	0.0	0.5	26.8	0.0	0.0	57.8

Name	α-T	α-T3	β-T	γ-T	β-T3	P8	γ-T3	δ-T	δ-T3	Total
Takara siyah Gülnar	6.1	13.0	0.0	1.8	0.0	0.6	37.8	0.0	0.0	59.4
Su üzümü Konya	3.0	14.3	0.0	1.3	0.0	0.3	35.1	0.0	0.0	54.1
Sergi Gülnar	5.3	11.4	0.0	0.6	0.0	0.9	33.4	0.0	0.0	51.6
Redglob murtici	15.5	33.7	0.0	6.5	0.0	1.6	33.2	0.0	0.0	90.5
Razaki beyaz Antep	8.7	36.8	0.0	2.3	0.0	1.1	17.4	0.0	0.0	66.3
Eksi kara Hadim	2.8	6.5	0.0	0.9	0.0	0.4	29.0	0.0	0.0	39.6
Toros beyazi Büyükeceli	2.9	17.6	0.0	1.2	0.7	0.9	31.7	0.0	0.0	55.0
Topacik siyah Konya	8.2	22.2	0.0	0.6	0.2	0.7	30.1	0.0	0.0	62.0
Karadimlit Taskent	7.2	13.0	0.0	3.5	0.0	0.6	52.8	0.0	0.0	77.2
Beylerce Denizli	26.4	23.9	0.8	3.3	0.4	2.1	43.3	0.0	3.0	103.2
Alyanak Denizli	22.0	19.1	0.0	3.5	0.0	2.2	29.2	0.0	0.0	76.0
Bogazkere Denizli	5.2	14.5	0.0	0.2	0.0	1.1	14.5	0.0	0.0	35.4
Alfons Denizli	10.0	10.3	0.0	0.5	0.0	0.9	18.4	0.0	0.2	40.4
Öküzgözü Denizli	20.8	14.4	0.0	2.3	0.4	1.0	21.2	0.0	0.7	60.7
Izazdag Denizli	9.0	13.5	0.0	2.4	0.0	1.5	17.4	0.0	0.4	44.2
Dimlit Denizli	11.6	15.3	0.1	1.3	0.0	1.7	35.6	0.0	0.8	66.3
Retglob Denizli	5.7	17.2	0.0	4.9	0.4	0.8	17.1	0.0	0.2	46.2
Soltaniye Denizli	13.7	13.9	0.0	4.0	0.0	3.1	20.4	0.0	0.2	55.3

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Short Communication

PHYSICOCHEMICAL QUALITY OF UNBRANDED BUTTER SOLD IN LAHORE

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ABSTRACT

The main objective of this study was to determine the quality of unbranded butter available in Lahore. Five different types of butter samples were collected from different regions of Lahore. Samples of butter were analyzed for fat, moisture content, fat free matter, refractive index iodine value, peroxide value, para anisidine value, totox value, total bacterial count and coliform count. Fat content of all the five samples were less than prescribed standards (minimum 84% fat), free fatty acids of sample D were 0.25% and peroxide value of all the samples ranged from 4.25-14.80 Meq./kg. Iodine value of A, D and E was 46.77, 38.50 and 40.50 respectively which indicated that samples were adulterated with vegetable oils. 60% of the samples were found positive for Coliforms and E.coli. From the results it can be concluded that quality of unbranded butter sold in Lahore is extremely poor and substandard from chemical and microbiological point of view.

1. Introduction

Butter is a dairy product made by churning fresh or fermented cream or milk. It is generally used as a spread and a condiment, as well as in cooking applications, such as baking, sauce making, and pan frying. Butter consists of butterfat, water and milk proteins [1]. Butter is very popular in Pakistan, there is a taste preference for solid fats in this part of world. Dairy sector in Pakistan is not well developed; 95-97% milk is sold in raw form. Thirteen milk processing plants are manufacturing milk and milk products. Small scale manufacturers and shop keepers manufacture and sale dairy products in extremely unhygienic conditions. Present study was planed to find out the physicochemical and microbiological quality of butter sold in Lahore Market.

2. Materials and methods

Butter samples of five different brands were purchased from milk shops and bakeries of Lahore and coded. Fat content of butter samples were determined by using Gerber method, moisture content was determined by drying the samples in air forced draft oven (Memmert, Germany) till constant weight and fat free matter was calculated from the difference of fat and moisture content by following the methods of [2]. Peroxide value, para anisidine value, totox value, iodine value free fatty acids and melting point was determined according to the methods of (A.O.C.S. 1990). Coliform and E.Coli were determined using VRBG and VRBL media by pour plate method according to the methods of [3].

3. Results and discussion

The mean values of fat content of different samples of unbranded butter sold in Lahore are presented in Table 1. The results showed that fat content of all the five samples (A, B, C, D & E) were less than prescribed standards of Pakistan Standard and Quality Control Authority (PSQCA) 84%. Fat content of all the samples varied significantly among each other, the lowest value with respect to fat was 72.5% was recorded in sample A. Moisture content of A, B and D was more than allowable limits of PSQCA (16% max.). The highest moisture contents 25.11% were recorded in sample A followed by 23.73% in D. The reason for higher moisture content in all the samples was probably be due to the lack of appropriate equipment for cream churning and butter working which may have contributed in this phenomenon. This might also be due to the urge of making more money. Sample D was criticized for having bad smell this was obvious from free fatty acids content (0.25%). The reason for high free fatty acids was due to the extremely poor handling of cream and the another reason for higher free fatty could be due to the use of foots of palm oil which is evident from the iodine value of sample D 38.50 as compared to other samples. Palm oil and other vegetable oils like canola oil is commonly used as adulterant in butter.

Table 1. *Physicochemical composition of butter samples*

Sample	FAT	M	FFM	FFA	PV	AV	TV	MP	IV	CF	EC
A	72.56^{e}	25.11^{a}	2.33^{a}	0.14	5.60	19.90	25.50^{d}	29.10^{d}	46.77	-ve	-ve
	± 2.89	± 1.61	± 0.11	$\pm 0.06^{d}$	$\pm 1.98^{d}$	$\pm 3.22^{d}$	± 3.66	± 3.82	$\pm 5.13^{a}$		
B	78.80^{c}	19.55^{c}	1.65^{c}	0.11	4.25	17.55	21.80	33.80	36.45	+ve	+ve
	± 3.25	± 1.23	± 0.08	$\pm 0.03^{e}$	$\pm 1.33^{e}$	$\pm 2.56^{e}$	$\pm 3.11^{e}$	$\pm 3.33^{c}$	$\pm 4.46^{d}$		
C	81.60^{b}	16.37^{d}	2.03^{b}	0.16^{c}	6.32	22.47c	28.79	34.60	35.62	+ve	+ve
	± 3.67	± 1.09	± 0.12	± 0.07	$\pm 1.70^{c}$	± 3.86	$\pm 3.77^{c}$	$\pm 3.92^{c}$	$\pm 4.10^{d}$		
D	75.20^{d}	23.73^{b}	1.47^{d}	0.25^{a}	10.69	30.67^{b}	41.36	38.50	39.84	-ve	-ve
	± 3.11	± 1.49	± 0.19	± 0.06	$\pm 1.99^{b}$	± 3.99	$\pm 4.26^{b}$	$\pm 3.99^{b}$	$\pm 4.76^{b}$		
E	82.50^{a}	15.22^{e}	2.28^{a}	0.19^{b}	14.80	39.77	54.57	40.50	38.11c	+ve	+ve
	± 4.59	± 0.98	± 0.14	± 0.02	$\pm 2.11^{a}$	$\pm 4.19^{a}$	$\pm 5.23^{a}$	$\pm 4.18^{a}$	± 4.22		

Means of duplicate experiment; means with same superscript letter in same column are statistically non significant by Tuckey's T-Test at 0.05 level of significance.

M: Moisture Content **FFM:** Fat Free Matter **FFA:** Free Fatty Acids **PV:** Peroxide Value
AV: Para Anisidine Value **TV:** Totox Value **MP:** Melting Point **IV:** Iodine Value
CF: Coliforms **EC:** E.Coli

The addition of vegetablie oils at higher levels is evident in sample A which has iodine value of 46.77. Iodine value of sample A indicated adulteration of vegetable oils in butter at fairly high level, which is also conform from the low melting point of sample (29.10 ^{0}C). Peroxide value of all the samples were more than recommended limits of PSQCA. The highest peroxide value was recorded in sample D (14.80 Meq./kg) followed by sample D (10.69 Meq./kg). Higher levels of peroxides of both the samples support the doubt of addition of foots of palm oil in the butter. Peroxide value of all the samples indicated the extremely poor quality cream was churned and the handling and storage conditions were extremely unhygienic and substandard. 60% of the butter samples were contaminated with coliforms and E.Coli. Free fatty acids and peroxide value of foots of palm oil is much higher than refined, bleached and deodorized palm oil [4]. Free fatty acids of butter should be less than 0.14% [1]. It was concluded that quality of unbranded butter available in Lahore is substandard and needs lot of improvement and consuming it could be hazardous for health.

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