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Pycnogenol® Augments Macrophage Phagocytosis and Cytokine Secretion

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Abstract: We previously reported that Pycnogenol®, procyanidins extracted from the bark of French maritime pine (*Pinus maritima* Aiton; the official botanical name is now *Pinus pinaster* Aiton) is a potent free radical scavenger. It has been shown to inhibit macrophage oxidative burst. Macrophages carry out their microbicidal and tumoricidal activities via oxygen-dependent and oxygen-independent mechanisms. The present study investigated the effects of Pycnogenol® on oxygen-independent killing mechanisms of macrophages, with particular interest in phagocytosis and cytokine release. J774 cells, a murine macrophage cell line, were preincubated with Pycnogenol® and then exposed to fluorescein-conjugated *Escherichia coli* particles for phagocytosis. Pycnogenol® significantly enhanced the phagocytosis by J774 cells. Incubation with Pycnogenol® resulted in a significant increase in cell size indicating macrophage activation. J774 cells were treated with Pycnogenol® for 22 hr and the supernatants were tested for the release of tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β). Pycnogenol® significantly increased the secretion of both TNF-α and IL-1β. These results suggest that Pycnogenol® can enhance the macrophage function by increasing its ability to phagocytosis and secretion of TNF-α and IL-1β. These two cytokines may provide costimulatory signals to enhance both the humoral and cellular immune responses to promote host defense.

Key Words: Pycnogenol®, J774 murine macrophage, phagocytosis, tumor necrosis factor-α, interleukin-1β

Introduction

Macrophages play an important role in host defense by phagocytizing foreign invaders, undergoing oxidative burst, presenting antigen, and secreting cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β). Macrophages are activated in inflammatory sites (Adams and Hamilton, 1984). They can be activated by bacterial lipopolysacchride, interferon-γ, IL-12, IL-18, ultraviolet irradiation and ozone (Adams and Hamilton, 1984; Vairo *et al*., 1992; Munder *et al*., 1998; Virgil *et al*., 1998). An increase in cell size, protein content, and enzyme activity has been used as markers of macrophage activation (Adams and Hamilton, 1984; Enane *et al*., 1993). The activated or "angry" macrophages as they are sometimes called, can efficiently destroy microbes, parasites, and tumor cells. Activated macrophages have been of interest in clinical studies in local or systemic adoptive cancer immunotherapy as they can act selectively against neoplastic cells (Bartholeyns, 1993). TNF-α, known for its cytotoxic effect on tumor cells, is secreted from activated macrophages and monocytes (Currie and Bashan, 1975). IL-1β, also termed lymphocyte activating factor, has been known to protect against lethal changes brought about by radiation, hyperoxia and infection (Gupta, 1988). Through TNF-α and IL-1β macrophages control the proliferation, differentiation, and effector functions of lymphocytes (Parslow and Bainton, 1997). TNF-α and IL-1β have overlapping biological activities that produce a broad range of effects on nonhematopoietic as well as hematopoietic cells (Gupta, 1988; Oppenheim and Ruscetti, 1997). The activation of macrophages is regulated by a network of mediators. For example, TNF-α and IL-1β enhance their own release as well as that of each other (Durum and Oppenheim, 1989). These two cytokines provide costimulatory signals to enhance the activation of helper T lymphocytes and thus promote both the humoral and cellular immune responses (Oppenheim and Ruscetti, 1997).

Pycnogenol® (PYC) is a blend of water-soluble oligomeric and monomeric procyanidins extracted from the bark of French maritime pine (*Pinus maritima* Aiton; the official botanical name is now *Pinus pinaster* Aiton) (Masquelier, 1987). It has been known to reduce inflammation, and alleviate a variety of conditions linked to the deleterious action of free radicals (Passwater, 1992; Passwater and Kandaswami, 1994).

In our laboratory, we have demonstrated that PYC can protect vascular endothelial cells from injury induced by an organic oxidant, *t*-butyl hydroperoxide (Rong *et al*., 1995). It can increase the levels of intracellular glutathione and enhance the activities of antioxidant enzymes (Wei *et al*., 1997). PYC also inhibits macrophage oxidative burst, lipoprotein oxidation, and hydroxyl radical-induced DNA damage (Nelson *et al*., 1998). We have shown that PYC enhances immune and hemopoietic functions and counters learning

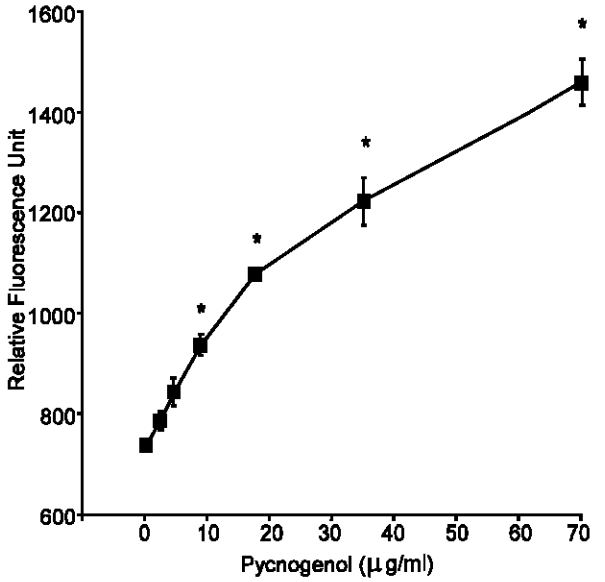

Fig.1: Effect of PYC on phagocytosis. J774 cells (10^5 cells/well) in 96-well plates were preincubated with PYC (2.18, 443, 8.75, 17.5, 35, and 70 µg/ml) in DMEM at 37 °C and 5% CO_2 for 2 hr. Following removal of PYC, fluorescein-conjugated *E. coli* particles were added and further incubated at 37 °C for 1 hr. Phagocytosis was measured with the fluorometric assay. Data represent means ± SE of triplicate samples. *Significant difference ($P < 0.05$) compared with control without PYC.

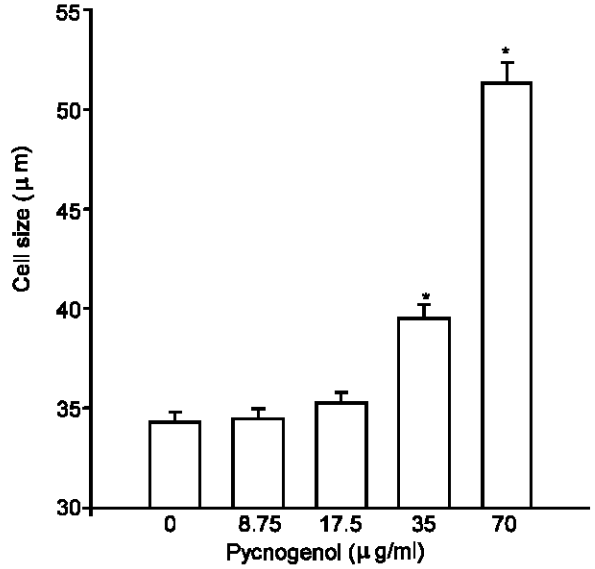

Fig. 2: Effect of PYC on cell size. J774 cells (7.4×10^5 cells/well) in 6-well plates were incubated with PYC (8.75, 17.5, 35, and 70 µg/ml) in DMEM at 37 °C and 5% CO_2 for 22 hr. Data represent means ± SE of three experiments. *Significant difference compared with control without PYC ($P < 0.05$).

impairment and memory deficit in senescence accelerated mice (Liu *et al.*, 1998; Liu *et al.*, 1999). More recently, we showed that PYC protects neurons from amyloid-β peptide-induced apoptosis (Peng *et al.* 2002). The clinical benefits of PYC have been summarized in a recent review article (Rohdewald, 2002). In the present paper, we report the effects of PYC on activation of macrophages pertaining to cell size, phagocytosis, and secretion of TNF-α and IL-1β.

Materials and Methods

Chemicals and Reagents: PYC (Pycnogenol® — a registered trade mark of Horphag Research Ltd., Geneva, Switzerland) was provided by Henkel Corporation (La Grange, IL, U.S.A.). Dulbecco's modified Eagles Medium (DMEM) and penicillin-streptomycin solution were purchased from Mediatech Co. (Herndon, VA, U.S.A.). Bovine calf serum (BCS) was from Hyclone Laboratories (Logan, UT, U.S.A.). Phosphate buffered saline (PBS) and citric acid were from Sigma Chemical (St. Louis, MO, U.S.A.). Fluorescein-conjugated *Escherichia coli* K-12 bio-particles were purchased from Molecular Probes (Eugene, OR, U.S.A.). Trypan blue was purchased from Matheson Coleman and Bell (Norwood, OH, U.S.A.). The Quantikine M mouse TNF-α Immunoassay kit was from R & D Systems (Minneapolis, MN, U.S.A.). PYC was dissolved in PBS. *E. coli* particles were suspended in PBS and sonicated before use. The bacterial number was determined visually by counting in a hemacytometer and adjusted to 10^8/ml.

Cell Line: The murine J774 macrophage cell line was obtained from the American Type Culture Collection (ATCC, Rockville, MD, U.S.A.). Cells were grown in DMEM, supplemented with 10% BCS, 200 U/ml penicillin and 200 µg/ml streptomycin, for 3-5 days, at 37 °C in a humidified 5% CO_2 atmosphere. The viability of cells used in experiments was always greater than 95% as determined by trypan blue exclusion.

Phagocytosis Assay: The microfluorometric phagocytosis assay was performed in 96-well plates as previously described (Wan *et al.*, 1993). Suspension of fluorescein-conjugated *E.coli* particles (100 µl/well) was used to measure the ability of macrophages to phagocytize these particles with or without PYC pretreatment. The results were expressed as the relative fluorescence unit.

Cell Size Measurement: J774 cells (7.4×10^5 cells/well) were seeded in 6-well plates and incubated with PYC (8.75, 17.5, 35, and 70 µg/ml) in DMEM at 37 °C for 22 hr. Random fields at 320 x magnification (Zeiss axiovert 100TV, Carl Zeiss Inc., Thornwood, NY, U.S.A.) were digitized (Hamamatsu C2400 CCD, Hamamatsu

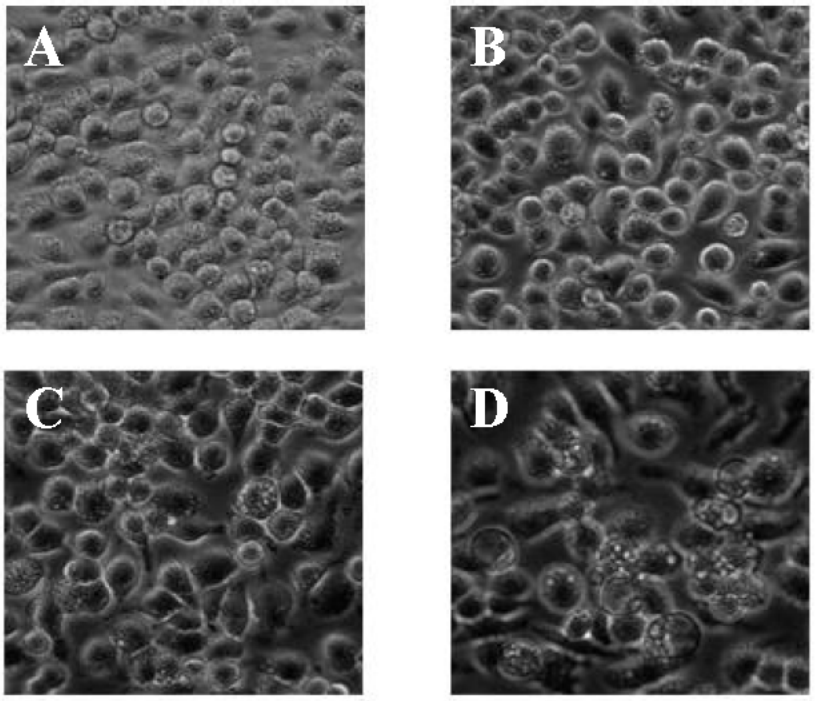

Fig. 3: Photomicrograph showing effect of PYC on cell size. Magnification 320 X. A, B, C, and D represent cells incubated with 0, 8.75, 35, and 70 µg/ml of PYC, respectively.

Corporation, Bridgewater, NJ, U.S.A.) for each experiment. Diameters of 60 cells at each PYC concentration were measured using the MetaMorph 2.5 software (Universal Imaging Corp., West Chester, PA, U.S.A.).

TNF-α Immunoassay: J774 cells (10^5 cells/well) in 96-well plates were incubated with PYC (8.75, 17.5, 35, and 70 µg/ml) in DMEM at 3 °C for 22 hr. The supernatant was collected from each well and the TNF-α levels were measured by using the Quantikine M mouse TNF-α Immunoassay kit following the manufacturer's instructions. The intensity of color of the final product is proportional to the amount of TNF-α. The absorbance was determined at 450 nm with 400 AT EIA (Whittaker Bioproducts, Walkersville, MD, U.S.A.).

Interleukin-1β: J774 cells (10^5 cells/well) in 96-well plates were incubated with PYC (8.75, 17.5, 35, and 70 µg/ml) in DMEM at 37 °C for 22 hr. The supernatant was collected from each well and stored at -20 °C until shipment. Determination of IL-1β level was performed by UMAB Cytokine Core Laboratory (Baltimore, MD, U.S.A.) using the biotin-strepavidin-peroxidase ELISA.

Statistical Analyses: Experimental data were analyzed with one-way analysis of variance (ANOVA) followed by Tukey's multiple range test for significant difference, and the results were expressed as means ± SE. Statistical significance was defined as $P < 0.05$. All statistical procedures were performed with Statgraphics software version 5.0 (STSC, Rockville, MD, U.S.A.).

Results

Each experiment was repeated at least three times with consistent results indicating that the assays used in this study were highly reproducible. Fig. 1 shows the effect of PYC on phagocytosis. PYC caused a concentration-dependent increase in the ability of J774 cells to phagocytize the fluorescein-conjugated *E. coli* particles as reflected by an increase of relative fluorescence units. Significant increases were noted between 8.75 and 70 µg/ml of PYC.

A concentration-dependent increase in cell size was observed when J774 cells were treated with PYC (Fig. 2 and 3). Statistically significant increases of 15% and 50% were noted with 35 µg/ml and 70 µg/ml of PYC, respectively (Fig. 2).

J774 cells were treated with PYC for 22 hr and the supernatants were tested for TNF-α release. A concentration-dependent increase of TNF-α release was noted with significant increases of 47 and 67% noted at 35 and 70 µg/ml of PYC, respectively (Fig. 4).

Fig. 5 shows the IL-1β release when J774 cells were treated with PYC. The IL-1β was below the level of detection up to 17.5 µg/ml of PYC. However, it increased significantly at PYC dosages of 35 and 70 µg/ml.

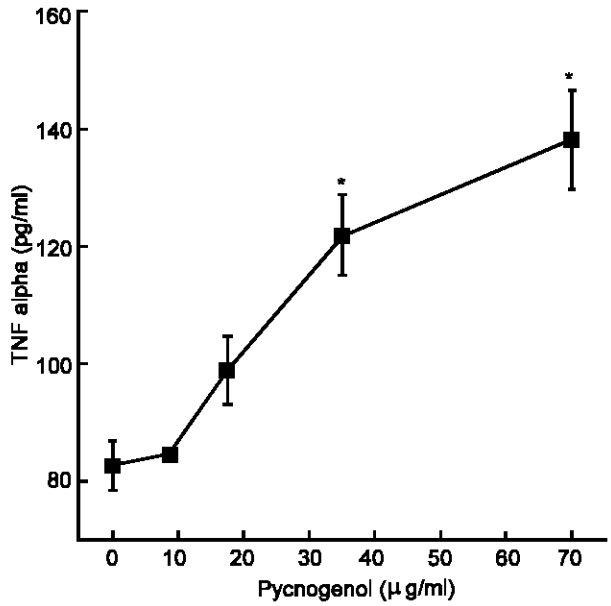

Fig. 4: TNF-α release by J774 cells. J774 cells (10^5 cells/well) in 96-well plates were incubated with PYC (8.75, 17.5, 35, and 70 µg/ml) in DMEM at 37 °C and 5% CO_2 for 22 hr. The supernatant was collected and the TNF-α release was measured. Data represent means ± SE of triplicate samples. *Significant difference compared with control without PYC ($P < 0.05$).

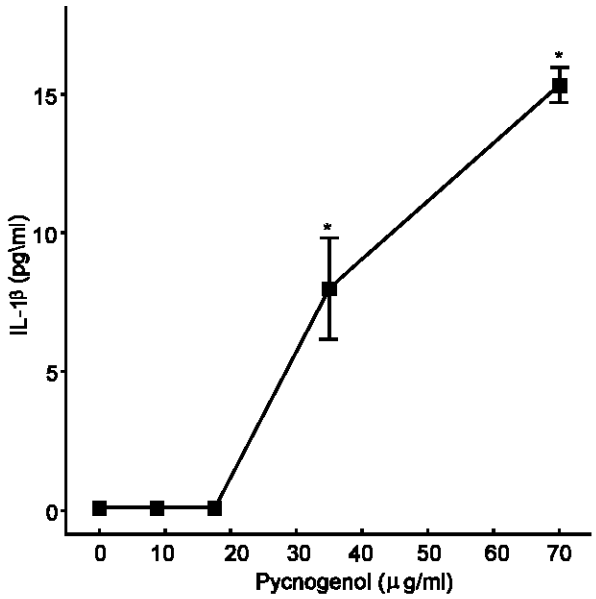

Fig. 5: Interleukin-1β release by J774 cells. J774 cells (10^5 cells/well) in 96-well plates were incubated with PYC (8.75, 17.5, 35, and 70 µg/ml) in DMEM at 37 °C and 5% CO_2 for 22 hr. IL-1β in the supernatant was measured. Data represent means ± SE of triplicate samples. *Significant difference from control without PYC ($P < 0.05$).

Discussion

Macrophages constitute a major part of the host defense system against infection and cancer. Macrophages carry out their microbicidal and tumoricidal activities by the oxygen-dependent killing via products of oxidative metabolism such as hydrogen peroxide, superoxide anion, and hydroxyl radical, and the oxygen-independent killing via cytokines and hydrolytic enzymes. We previously reported that PYC inhibited generation of reactive oxygen species such as peroxides in macrophages (Bayeta and Lau, 2000) indicating its ability to abolish the oxygen-dependent killing mechanism. This inhibition is used to explain the anti-inflammatory property of PYC reported in the literature (Blazso *et al.*, 1994; Blazso *et al.*, 1997). Our curiosity to find out whether or not PYC affected the oxygen-independent killing mechanism prompted us to investigate the effects of PYC on macrophage phagocytosis and cytokine secretion. TNF-α and IL-1β were chosen for our investigation because these two cytokines play an important role in regulating normal immune functions. Data from this study indicate that PYC did not inhibit macrophage phagocytosis and secretion of TNF-α and IL-1β. On the contrary, incubation of macrophages with PYC resulted in a greater phagocytic activity and increased levels of TNF-α and IL-1β. Thus, even though our previous study showed PYC to cripple oxygen-dependent killing mechanism, it is intriguing that the present study demonstrates PYC's ability to enhance phagocytosis and cytokine secretion—a part of the oxygen-independent killing mechanism of macrophages.

An increase in cell size is one of the earliest characteristics observed in activated macrophages and is related to the degree and rate of cell spreading; activated cells spread more rapidly and to a greater extent than the resident cells (Adams and Hamilton, 1984; Enane *et al.*, 1993). We observed a significant increase in cell size with PYC indicating its ability to activate macrophages. Activated macrophages exhibit a greater phagocytic activity than the resident cells (Zelikoff *et al.*, 1991). In our study, PYC-treated macrophages indeed exhibited a greater phagocytic capability than the untreated cells. With as little as 8.75 µg/ml of PYC, the phagocytosis increased by 27% as compared with the untreated control cells (Fig. 1). Activated macrophages are known to increase their secretion of hydrolytic enzymes and cytokines. We did not investigate hydrolytic enzymes in the present study. Previous studies have, however, demonstrated PYC's ability to enhance the activity of enzymes in endothelial cells and

macrophages (Wei *et al*., 1997; Bayeta and Lau, 2000). The present study shows a significant increase of TNF-α and IL-1β secretion when macrophages were incubated with PYC. These two cytokines are unique in that they enhance their own release as well as that of each other (Durum and Oppenheim, 1989). In addition, they provide costimulatory signals to enhance the activation of helper T- lymphocytes and thus promote both the humoral (B-lymphocytes) and the cell-mediated (T-lymphocytes) immune responses (Oppenheim and Ruscetti, 1997).

Several studies have demonstrated that PYC is a potent anti-inflammatory phytochemical (Blazso *et al*., 1994; Blazso *et al*., 1997; Bayeta and Lau, 2000). PYC differs from currently used steroidal or non-steroidal anti-inflammatory drugs in that it does not exhibit any adverse side-effects (Bayeta and Lau, 2000). Unlike most anti-inflammatory drugs that exert immunosuppressant activity against B- and T-lymphocytes, the present study shows PYC's ability to increase cytokines known to promote immune functions. Cheshier *et al*. (1996) used retro virus and chronic ethanol intoxication to induce immunosuppression in mice. Feeding with PYC delayed the development of immune dysfunction in this animal model. PYC restored the imbalanced cytokine secretion by T-helper 1 and T-helper 2 cells, which are important in cellular and humoral immunity, respectively. In our study with senescence-accelerated mice, we demonstrated that PYC can restore the immune and hemopoietic functions that are altered in the aging process. Feeding animals with PYC significantly improved the severely depressed B- and T-lymphocyte responses to mitogens (Liu *et al*., 1999). These studies suggest that PYC can enhance B and T cell function and at the same time it can exert potent anti-inflammatory effects.

In conclusion, the data from this *in vitro* study indicate that PYC is capable of activating macrophages to enhance phagocytosis and the secretion of TNF-α and IL-1β. These results suggest that PYC may play an important role in modulating the immunological function by means of macrophage activation. PYC has been shown to have potent anti-inflammatory property. Unlike anti-inflammatory drugs currently in use, PYC is a novel and unique therapeutic agent in that it does not exhibit immunosuppressant activity. Whether or not these beneficial effects occur in humans warrants further clinical studies.

Acknowledgments

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Innovations in the Traditional Kunun Zaki Production Process

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Abstract: The traditional kunun zaki production process has been improved upon. The usual 24 hours process has been shortened to a maximum of 12 hours. Steeping of the grains in warm water with 5% sodium metabisulphite help in softening them. Liquefaction and saccharification of gelatinized sorghum starch with sweet potato and *Cadaba farinosa* crude extracts did not increase the amount of reducing sugar after 6 hours of incubation. Similarly, the specific density of the slurry remained the same after 6 hours of incubation with the crude enzyme extracts. Due to shortened saccharification process, the nutrient content of kunun zaki from improved process was a bit higher. The protein content was 5.4 and 4.1% for the improved and traditional processes respectively. The product from improved process was preferred to that in the traditional process.

Key Words: Sorghum starch, saccharification process, *Cadaba farinosa*

Introduction

Kunun zaki is a cereal-based beverage in Nigeria. The cereals utilized in its production are millet, sorghum and maize in decreasing order of preference (Gaffa *et al*., 2002a). Sometimes, the cereals could be used in composite form in its production but this is more common with only millet and sorghum grains. The preferred ratio of mixing is 1:2 (w/w) sorghum/millet. The traditional production process involves steeping the grains in local household utensils such as buckets, drums, calabashes or earthenware vessels (Adeyemi and Umar, 1994). The steeping duration depends on the cereal used but may vary between 12 and 72 hours for millet/sorghum and maize respectively (Gaffa, 2000). Grinding of the steeped grains mixed with spices (ginger, clove, red and/or black pepper) is done with local milling machines and part of the slurry (3/4 volume) is gelatinized with boiling hot water (Onuorah *et al*., 1987a). The remaining part of the slurry (1/4 volume ungelatinised) containing liquefying agents (sweet potato tuber paste, malted rice, extract of *Cadaba farinosa* stem) is mixed with the gelatinized portion when the temperature is about 60 – 70 ^{o}C. The mixture is altogether left overnight at room temperature for chance fermentation and filtered using local sieve the next morning. The filtrate-kunun zaki is consumed as a beverage with or without addition of sugar as a sweetener. The whole process lasts about 24 hours. The nutrient content and microbiological quality of this product has been reported (Gaffa *et al*., 2002b and c; Onuorah *et al*., 1987b). The consumption rate of the beverage has also been studied (Gaffa *et al*., 2002a). Owing to the high demand for this product and the high consumption rate, it is thought that the present traditional production process is outdated, inefficient, time consuming and with product quality varying between batches. In this present study, attempts have been made to improve on the traditional production process with the hope of maintaining nutrient and improving microbiological quality of the final product. The nutrient and sensory qualities of kunun zaki from the new process has been analyzed and compared with the traditional process.

Materials and Methods

Materials: *Sorghum bicolor*, dry sweet potato chips (*Ipomea batatas*) and ginger (*Zingiber officinale*) were all bought in bulk in Bauchi Central market, Nigeria. All items were bought in their standard measures as offered for sale. The ratio of ingredients used is presented in Table 1

Preliminary investigation: Attempts were made to find out whether the long period of chance fermentation was really necessary or whether the process comes to a halt after some time. Crude extracts of sweet potato and *Cadaba farinosa* were used on gelatinized starch. Two methods were employed in determining the termination time: the specific density method and estimation of reducing sugar. In the specific density determination, 30 ml of gelatinized (2% w/v) starch were dispensed in eight clean beakers each. To this was added 0.25% (v/v) of extracts from *C. farinosa* and dry sweet potato separately in each case. The preparations were in triplicate and allowed to stand in water bath at 60 ^{o}C for 0, 1, 2, 3, 4, 5, 6 and 7 hours respectively. At the end of each period, the specific density of each was determined using the method of Giese (1995).

Table 1: Kunun zaki beverage formulation

Ingredients	Quantities (g)
Sorghum bicolor	1346
Dry sweet potato chips	100
Ginger	16
Kunun zaki (ml)*	8963*

As final volume of the beverage.

Table 2: Nutrient content of kunun zaki produced by improved production process and the traditional process

Constituents	Nutrient contents	
	Improved	Traditional
Moisture	89.77	91.54
Crude protein	5.39	4.48
Crude fat	0.32	0.34
Ash	1.30	1.22
Carbohydrate	3.22	2.42
pH	5.43	4.92
Total soluble solids	7.50	6.00

Table 3: Sensory evaluation scores for kunun zaki samples produced by the improved method and the traditional process

Parameters	Sample codes with scores	
	AIM	TRD
Colour	23	11
Sweetness	29	5
Flavour	25	9
Mouth feel	26	8
Overall acceptability	29	5

AIM – Kunun zaki produced by the improved method TRD – Kunun zaki produced by the traditional method

Measurement was carried out gravimetrically using a specific density bottle and compared with equal volume of water at the same temperature. In the estimation of reducing sugar, extracts from *C. farinosa* and dry sweet potato were also used. In each case, 100μl of each extract was added to 1000μl gelatinized (1% w/v) sorghum starch in eight different test tubes. This was incubated as in specific density determination. The reducing sugar produced in each case was determined using Somogyi's colorimetric method (1952).

Production processes for kunun zaki

Traditional process: The traditional process followed is that which is common in Bauchi and Gombe states of Nigeria already reported (Gaffa *et al.*, 2002b) and is outlined in Fig. 1. Ingredients used were as in Table 1.

Improved production process: The same ingredients as presented in Table 1 were employed in this process. However, the washing was more thorough. The grains were steeped in clean warm water (60-70 ^{o}C) in ratio 1:2 (v/v) using a sterile beaker. To the steeped grains, 0.5% (w/w) sodium metabisulphite was added. The steeped

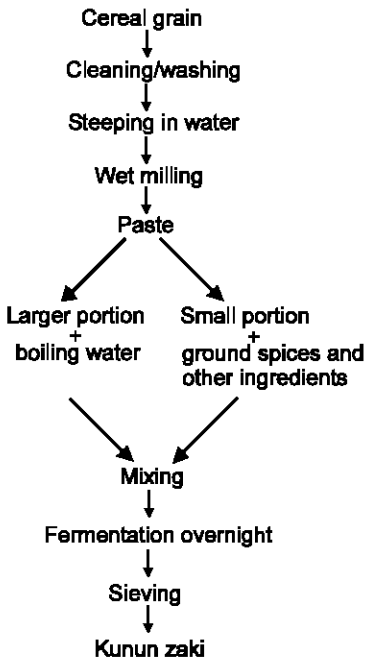

Fig. 1: Flow diagram of traditional kunun zaki production in Bauchi and Gombe State, Nigeria

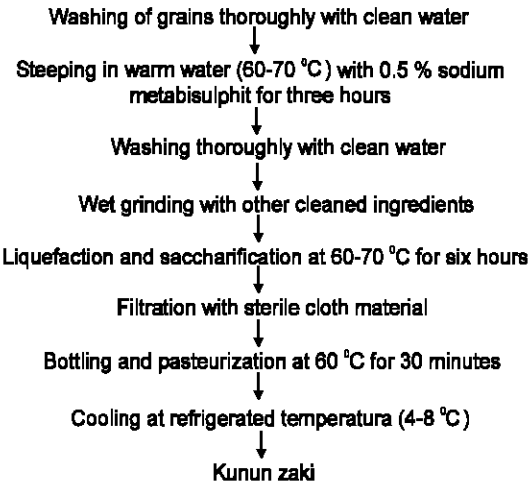

Fig. 2: Improved kunun zaki production process

grains and sweet potatoes were allowed to remain in the water for three hours. The soaked grains were removed from the water and washed again with fresh water. It was then ground to paste with a disc attrition-grinding machine that had earlier been washed thoroughly. The paste was divided into two unequal parts in ratio 1:3 (v/v). Boiling hot water was added to the larger portion to gelatinize. When the temperature dropped to 65-70 ^{o}C, the smaller portion was added. This was stirred using a sterile glass rod and allowed to stand at room temperature (25-28 ^{o}C) for six hours to effect liquefaction. The mixture was filtered at the end of

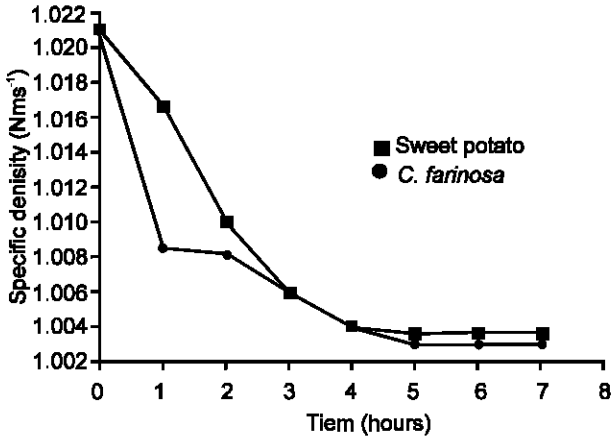

Fig. 3: Termination of starch liquefaction and saccharification by *C. Farinosa* and sweet potato extract (2 % w/v) on gelatinised sorghum starch

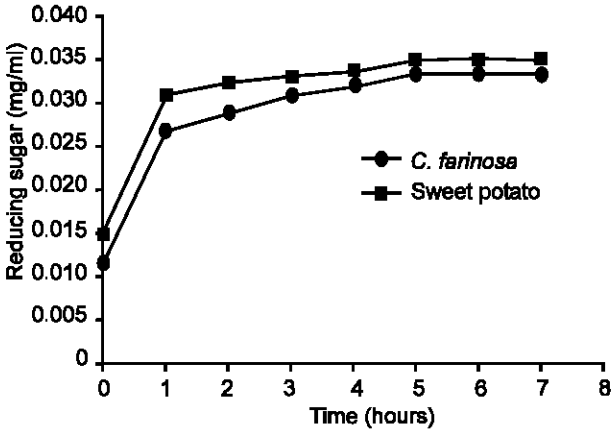

Fig. 4: Termination of starch liquefaction and saccharification by C. Farinosa and sweet potato extract (2% w/v) on gelatinised sorghum starch

the six hours using a muslin cloth that was boiled to sterilize. The improved method is outlined in Fig. 2.

Nutrient qualities of kunun zaki samples produced by the traditional and improved methods: The kunun zaki obtained from this process (improved) was compared in its nutritional quality (using the standard methods of Association of Official Analytical Chemists AOAC, 1984) and sensory attribute with that produced by the traditional process. The paired comparison test described by Ihekoronye and Ngoddy (1985) was adopted in sensory evaluation of the products.

Results

The effect of starch liquefaction on specific density of gelatinized sorghum starch: Fig. 3 shows that the specific density of the gelatinized starch decreased as a result of liquefaction brought about by the addition of *C. farinosa* and sweet potato extracts. The result also showed that the specific density decreased as the incubation time increased for both extracts. There were no decreases after the 5th hour in all the extracts.

Termination in the production of reducing sugar during incubation with liquefying agents: Fig. 4 showed the termination time in the amount of reducing sugar produced during liquefaction of gelatinized sorghum starch. Generally, optimum production of reducing sugar was obtained within the first 60 min (Fig. 4) after which there was no appreciable change in total reducing sugar with both *C. farinosa* and sweet potato. However, a higher peak was observed for sweet potato. The increase in number of hours of incubation caused slow but progressive increase in the amount of reducing sugar up to the sixth hour. No further increase was noticed after this period. The traditional kunun zaki production process takes not less than 24 hours before the final product is obtained. This wastes a lot of time and gives room for contaminants to get into the product. Based on our findings liquefaction takes not more than 6 hours.

Nutrient qualities of kunun zaki produced by traditional and improved methods: The nutrient contents of kunun zaki produced by the traditional and improved methods are presented in Table 2. Moisture content was slightly higher (91.54) in the traditional product compared to the improved product. Generally, the values for all the parameters were similar except for pH that was lower (4.92) in the traditionally produced kunun zaki product compared to that in the improved (5.43).

Sensory comparison between kunun zaki samples from the improved and the traditional process: In Table 3, average scores from the 34 member panelists on the sensory paired comparisons between the kunun zaki samples produced by the improved method and that produced by the traditional method is presented. In all the parameters assessed, the sample produced by the improved method had the highest scores. However, statistical analysis showed that there was no significant difference at 5% in colour between the samples. With the rest of the parameters (sweetness, flavour, Mouth feel, and overall acceptability) there were significant differences. Majority of the judges preferred the sample from the improved production process to that from the traditional process.

Discussion

Effect of liquefaction on specific density and production of reducing sugars from gelatinized sorghum starch. The progressive increase in the amount of reducing sugars with time (Fig. 4) indicates that the enzyme activity at the start of the reaction was fast resulting in increase of reducing sugars in the beverage. This trend is similar to the reduction in the specific density of the beverage (Fig. 3) that decreases with time. The reason why there was no further increase in reducing sugar with time after the 6th hour may be that the beta amylase enzyme for the hydrolytic process had completed the reaction. It could also be that the bonds of the starch

molecules left unattached are not susceptible to the enzymes. This may also be the reason for the leveling of the graph (Fig. 3) after five hours during the specific density determination. Generally, the hydrolysis of starch is accomplished by two stages depending on the nature of bonds holding the molecules. The initial rapid and random attack on the substrate leads to rapid loss in viscosity. The second stage involves the hydrolysis of alpha-1, 4-glycosidic bonds by the beta amylases yielding reducing sugars. This view agrees with Karel (1975). The slight differences in nutrient contents of the kunun zaki produced by the improved method and that through the traditional process (Table 2) were due to slight variations in the production process. The low pH value of 4.92 observed for kunun zaki from the traditional process could be due to the metabolic activities of contaminating microorganisms particularly lactic acid bacteria that play a role in the production process. Maintenance of hygienic conditions in the laboratory may have caused the pH of the improved beverage to be higher (5.3). The protein content (5.4%) of the beverage from the improved processing was higher than that (4.1%) in the traditional process. This proves that when the carbohydrate content of a food is nearly depleted, the nitrogenous compounds are metabolized by microorganisms in the product and agrees with Brown and Booth (1991). It could also be that the level of contamination was so high leading to this reduction in protein content. The contamination, probably with extended liquefaction process in the traditional process has affected the moisture content. The enzymes from liquefying agents and the metabolic activities of contaminants seem to have continued hence larger molecules could have broken down leading to these changes. The drop in total soluble solids is also linked to metabolic activities of the contaminants. The introduction of sodium metabisulphate and the shortening of liquefaction process to 6 hours is the reason for reduction in the level of contaminants. The reduction in level of contaminants leads to maintenance of high nutrient content in the beverage from the improved process. The lack of significant statistical difference in the sensory scores for colour (Table 3) of improved and traditional kunu zaki shows that colour variation does not affect acceptability of the bevaerage. The high total soluble solids (TSS) value (7.50%) of the beverage from the improved process and the pH value of 5.43 were thought to have influenced the kunun zaki acceptability. The TSS imparts sweet taste to the beverage and the pH a less harsh or slightly acidic taste to the food. In the improved production process of kunun zaki outlined in Fig. 4, the complete deviation from the traditional process was avoided. This was for the purpose of maintaining flavour, taste and other characteristics that are known to be peculiar with this beverage so that consumers will not reject it. It is known that if foods are not prepared according to individual's likes or dislikes, either small quantities of the product will be consumed or the disliked food rejected. This view agrees with Adams and Erdman (1988).

It is concluded that Kunun zaki can be produced within a shorter period than the usual 24 hours. The liquefaction process may take just 6-7 hours to be completed. This reduces the chances of more contaminants in the food as well as the risk of food borne infections. Moreover the nutrients are maintained under such conditions and the products are also acceptable to all consumers.

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The Effect of Soaking and Cooking on the Oligosaccharide Content of Seker a Dry Bean Variety (*P. vulgaris, L*) Grown in Turkey

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Abstract: In this research, the effects of cooking alone, soaking-cooking combination on the oligosaccharide contents of a registered dry bean variety, Seker grown in Turkey was studied. Saccharose, raffinose and stachyose contents of the raw material were 3.91, 1.86 and 3.84%, respectively. The highest removing, to the extent of decrease up to, approximately, 70% was achieved by soaking in 0.5 % sodium bicarbonate solution for 18 hour followed by cooking in pressured kettle. These conditions could be recommended to remove undesirable sugar contents of the Seker bean used for culinary purposes.

Keywords: Dry beans, oligosaccharides, flatulence, soaking, cooking

Introduction

Legumes, especially beans are considered important and inexpensive protein and dietary fiber sources in human nutrition. Further, beans contain a considerable amount of vitamins and minerals (Vidal-Valverde *et al*., 1993). All legumes, however, contain several oligosaccharides, among which raffinose, stachyose and verbascose are of importance (Naczk *et al*., 1992; Abdel-Gawad, 1993; Trugo *et al*., 1993). These sugars are generally undesirable due to flatus factors. Because human alimentary tract is deprived of ∝-galactosidase capable of hydrolyzing the ∝- 1→6 galactoside linkage, therefore, these oligosaccharides are not digested and accumulate in large intestine where they are undergone anaerobic fermentation by bacteria. Thus, some gases are produced owing to fermentation, such as carbon dioxide, hydrogen and methane (Reddy *et al.,* 1980; Fleming, 1981; Alfred *et al*., 1982).

Traditional treatments such as soaking, cooking, germinating and fermenting have been used to improve nutritional quality of the legumes, the dry beans (Trugo *et al*., 1993; Barampama and Simard, 1994). It has been shown that, antinutritional and flatus factors are removed considerably by utilizing these processes. Although there are a number of research associated with oligosaccharides of dry beans, unfortunately, there is a little research about the oligosaccharides of the dry beans grown in Turkey and the removal procedures before the consumption. The aim of this research were to determine oligosaccharide contents of registered dry bean variety, Seker, grown in Turkey, and to see what extent of some treatments like soaking and cooking are effective in the removal of some oligosaccharides.

Materials and Methods

Dry bean variety Seker samples used as research material were supplied from East Anatolia Institute of Agricultural Research. The damaged beans were segregated from main population, and then raw bean material were mixed with distilled water at 1:3 (w/v) and soaked at room temperature (20±2 °C) for 12 or 18 hours. Alternatively, soaking was undertaken in the same conditions in which distilled water replaced by 0.5% sodium bicarbonate solution. After the draining the soaking water and washing, the beans were mixed with distilled water in 1:4 (w/v) ratio and cooked in boiling water (conventional cooking) for 60 minutes or in a pressured kettle (121 °C- 15 psi) for 30 minutes.

Chemical Analyses: Total solids, ash, protein contents and pH of the samples were determined according to AOAC (Anonymous, 1975), while the determination of starch amount was done according to (Anonymous, 1983).

Extraction and identification of oligosaccharides: Raw and soaked-cooked dry beans were subjected to oligosaccharide extraction with 100 ml (80%) ethanol according to Tanaka *et al.* (1975). The extracts and washings were combined and concentrated to 100 ml under vacuum at 50 °C. The oligosaccharides were separated and identified by thin-layer chromatography (Tanaka *et al*., 1975), while the sugars (raffinose and stachyose) were identified by comparison with the reference standards obtained from Nestec (S.A. Centre de Recherche, Sweden) and saccharose from Fluka (Fluka Chemie AG CH-9470 Buchs).

Quantitative analysis of oligosaccharides: Saccharose, raffinose and stachyose contents of the beans were quantitavely determined by using guide-strip technique. Sugar spots on chromatograms were scraped off and extracted with 2 ml of distilled water in a test tube overnight at room temperature (Tanaka *et al*., 1975). Then oligosaccharides were determined with thiobarbutiric acid reaction (Percheron, 1962) with the reference standards containing 10-100 µg/ml of each oligosaccharide.

Table 1: The results of chemical properties of raw Seker dry bean variety (%, dry bases)

Dry matter	Moisture	Protein	Starch	Ash	pH	Total oligosaccharide
90.39	9.61	20.11	38.21	4.11	6.45	5.69

Table 2: Mean Sugar Contents and SD values of Seker Bean Variety, (%, Dry Bases)

process	sugar									
	Sucrose				Raffinose			Stachyose		
	Mean	SD	Δ		Mean	SD	Δ	Mean	SD	Δ
A*	3.91 ±	0.06	-		1.86 ±	0.24	-	3.84 ±	0.69	-
B	3.37 ±	0.01	(%14)		1.52 ±	0.11	(%18)	3.12 ±	0.22	(%19)
C	2.88 ±	0.06	(%26)		1.16 ±	0.22	(%38)	1.55 ±	0.45	(%59)
D	3.04 ±	0.30	(%22)		1.08 ±	0.04	(%42)	2.74 ±	0.13	(%29)
E	2.73 ±	0.06	(%30)		0.69 ±	0.06	(%63)	1.93 ±	0.01	(%50)
F	2.90 ±	0.02	(%26)		0.94 ±	0.03	(%50)	1.87 ±	0.08	(%51)
G	2.59 ±	0.11	(%34)		0.63 ±	0.03	(%66)	1.85 ±	0.08	(%52)
H	2.86 ±	0.09	(%27)		0.86 ±	0.01	(%54)	1.62 ±	0.69	(%58)
I	2.27 ±	0.40	(%42)		0.79 ±	0.35	(%58)	1.87 ±	0.05	(%52)
J	2.07 ±	0.20	(%47)		0.74 ±	0.04	(%60)	1,16 ±	0.06	(%70)
K	1.38 ±	0.21	(%65)		0.53 ±	0.10	(%72)	1.15 ±	0.28	(%71)

SD: standard deviation, Δ: decrease in percentages

A*= raw; B= cooked in boiling water (60 min); C= pressured cooking (121 °C, 15psi) for 30 min; D= soaked in distilled water 12 hr and cooked in boiling water (60 min); E= soaked in distilled water 18 hr and cooked in boiling water (60 min); F= soaked in 0.5% $NaHCO_3$ solution 12 hr and cooked in boiling water (60 min); G= soaked in 0.5% $NaHCO_3$ solution 18 hr and cooked in boiling water (60 min); H= soaked in distilled water 12 hr and pressured cooking (121 °C, 15psi) for 30 min; I= soaked in distilled water 18 hr and pressured cooking (121 °C, 15psi) for 30 min; J= soaked in 0.5% $NaHCO_3$ solution 12 hr and pressured cooking (121 °C, 15psi) for 30 min; K= soaked in 0.5% $NaHCO_3$ solution 18 hr and pressured cooking (121 °C, 15psi) for 30 min

The research was conducted in two replications, and statistical significance of means was evaluated by analysis of variance (Yildiz and Bircan, 1991).

Results and Discussion

The results of the chemical analyses of the raw Seker dry bean variety were presented in Table 1.

As seen in Table 1, moisture and protein contents of the bean were 9.61 and 20.11% respectively, but these values were reported as 8.52 and 16.36% for the same bean (Guvenc and Gungor, 1996). The differences seen in this variety are usually due to the several factors such as ecological aspects; soil quality, cultivation techniques, maturity levels, transportation and storage conditions (Cemeroglu and Acar, 1986).

The processes applied in this research on the Seker dry bean variety and the changes occurred in saccharose and oligosaccharide contents were shown in Table 2.

As could be noticed in Table 2, cooking the samples after soaking in sodium bicarbonate solution causes more decrease in the sugar contents than that of the samples soaked in distilled water. Similar results were also reported that legumes soaked in alkali medium gave lower sugar content compared to other soaking conditions (Abdel-Gawad, 1993). Also, Ku *et al.* (1976) noted that soaking in the 0.5% sodium bicarbonate solution might increase softening of the testa and cotyledons that could increase the sugars extraction.

In this research, pressured cooking was significantly ($p<0.01$) more effective on the sugar degradation than that of the conventional cooking. The decrease in stachyose content was high in the pressure-cooking samples that were not soaked (Table 2). However, it was reported that sucrose and oligosaccharides contents of the legumes with pressured cooking were lower than that of the normal cooking conditions (Jood *et al.*, 1985). It was speculated that the cotyledon is lightly damaged with pressured cooking and may absorb more water (Reddy and Salunkhe, 1980).

In this research, the variation of among the soaking times was significant ($p<0.01$), for example, when the soaking time increased, the sugar contents of the samples were decreased. Similar results were also reported by some other researchers (Kataria *et al.*, 1990).

As a result, pressure cooking after soaking in distilled and sodium bicarbonate solutions for 18 h decreased the following sugars; saccharose, raffinose and stachyose contents at 42-65, 58-72 and 52-71%, respectively. Although the beneficial effects of soaking in

sodium bicarbonate solution to decrease saccharose and oligosaccharide contents, alkali condition may cause further destruction in the Vitamin B contents, especially thiamin and riboflavin (Swaminathan, 1974). Therefore, tap water might be a good alternative to protect vitamins and have a moderate decrease for the flatulence factors.

Acknowledgment

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Evaporation Rate of Moisture from Dahi (Yogurt) During Storage at Refrigerated Condition

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Abstract: Different types of dahi were prepared by using banana juice and the prepared dahi samples were divided into two group having all types of dahi in each group. One group was covered with paper on the top of the cup and another group was without covered and were kept inside the refrigerator having a temperature of approximately 50 °C for a 16 days storage period. The removal of moisture percentage was measured every four days of interval during storage. The evaporation rate (gm/d) of dahi samples was also calculated by how much amount of water was removed per day during storage. The removal of moisture percentage and evaporation rate (g/d) of dahi samples of with cover group was lower than that of dahi samples of without cover group and evaporation rate of dahi samples of both with cover and without cover group was increased gradually due to incorporation of banana juice.

Key Words : Evaporation rate, fermented milk, plain dahi, banana juice mix dahi

Introduction

One of the most popular and oldest fermented milk product in Indian subcontinent is dahi or yogurt which results from lactic fermentation of milk. People of all ages like it. It is very healthy and nutritious dairy product. It is valued for controlling the growth of bacteria and incuring intestinal disease like constipation, diarrhoea and dysentery (Shahani and Chandan, 1974). Anticarcinogenic effect of dahi has been reported by Ayebo and Shahani (1980). Dahi or yogurt is also effective in lowering the blood cholesterol. Arsenic poisoning may be cured by taking dahi (Annonymus, 1997). In Bangladesh it is a common practice to store dahi in refrigerator after preparation. Usually the humidity percentage of refrigerator is lower than that of the normal humidity of air. For this reason food materials kept in the refrigerator loss weight due to the dehydration of water. But we do not know exactly how much water is removed from the dahi and what is the evaporation rate when dahi is kept at refrigeration condition. This information is very important for the people who are involved in milk products (like dahi) business and sale their products on the basis of weight. Because, when they keep dahi in the refrigerator for several days, the product (dahi) loss its weight, which is undesirable to a businessman. No research work has yet been done in our country in this line. For this reason, this piece of research work was undertaken to get some idea about the evaporation rate(gm/d) and removal of moisture percentage of dahi when they are stored in the refrigerator and also to know the effect of using cover on the cup of dahi during storage.

Materials and Methods

This experiment was conducted at the Dairy Science and Poultry Science Laboratory of Bangladesh Agricultural University during the period from 1st July to October 30th 2000. The following steps were taken in conducting the study.

Whole milk was collected from Bangladesh Agricultural University, Mymensingh, Dairy Farm. Juice from the banana fruit (*Musa sapientum*) was prepared in the laboratory and kept in the refrigerator. Whole milk was boiled in a pan for some times to reduce about 20-25% of its original volume. Sugar was added to the milk at the rate of 10% during boiling. During heating milk was stirred thoroughly with the help of a stirrer. After desired heating, milk pan was taken out from the heater and allowed to cool. Banana juice was taken out from the refrigerator and kept in the room temperature for melting. When the temperature of milk became about 37 °C then the milk was divided into four equal portions. For the preparation of different types of dahi, banana juice was added into each portion of milk in a following proportions:

Banana juice (Musa sepientum) 10% denoted as A type dahi. Banana juice (Musa sepientum) 20% denoted as B type dahi. Banana juice (Musa sepientum) 30% denoted as C type dahi.

Table 1 :Comparison of average evaporation rate (gm/d) of dahi samples of with cover and without cover group

Parameters	10% level of banana dahi (A)	20% level of banana dahi (B)	30% level of banana dahi(C)	Plain/ Control dahi (D)	Level of significance
Without cover group (gm/d)	1.40± 0.001	1.48± 0.001	1.55 ± 0.001	1.33± 0.001	**
With cover group (gm/d)	1.05± 0.001	1.20± 0.001	1.28± 0.001	0.96± 0.001	**

NS = Non significant ** = Significant at 1% level A= 10% banana dahi B= 20% banana dahi
C= 30% banana dahi D= Plain/control dahi

Table 2 : Comparison of removal of moisture percentage of dahi samples of with cover and without cover group

Parameters	10% level of banana dahi (A)	20% level of banana dahi (B)	30% level of banana dahi(C)	Control / plain dahi (D)	Level of significant
Without covered	83.20^{b}±1.01	84.61^{b}±1.01	87.95^{a}±1.01	82.90^{b}±1.01	**
With covered	62.79^{c}±0.47	68.49^{b}±0.47	72.52^{a}±0.47	59.98^{d}±0.47	**

NS = Not significant ** = Significant at 1% level A= 10% banana dahi B= 20% banana dahi
C= 30% banana dahi D= Plain/control dahi

Banana juice (Musa sepientum) 0% denoted as D type dahi (plain/control dahi).

Each portion of milk was inoculated with desirable proportion of culture (2%) which was collected from local market. The anato color (seed) was incorporated into different portions of milk. Samples from each portion were taken in a different plastic cups. The samples were incubated at 37 ^{0}C until the complete coagulation (8-12 hrs). After completion of the coagulation the dahi samples (A, B, C, D type) were divided into two group having all four types of dahi in each group. One group of dahi samples were kept at refrigeration temperature (5 ^{0}C) without using any cover on the top of the cup and another group of dahi samples were kept at same refrigeration temperature by using cover with paper on the top of the cup for a 16 days of storage period. From a previous study it was found that plain dahi could kept at refrigerated condition for consumption up to 16 days. Here, 16 days period was selected on the basis of previous findings. The samples were weighted initially and there after every four days interval there weights were taken to calculate the loss of moisture during storage due to evaporation.

All experiment materials were completely homogenous and statistical analysis was done as per Steel and Torrie (1980) by using Completely Randomized Design. Analysis of variance test was done to find out the statistical difference between the treatments. In case of significant difference, the difference among treatment means were compared by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

Results and Discussion

Evaporation rate (gm/d): The evaporation rate (gm/d) of different types of dahi samples during the storage period in the refrigerator are shown in Table 1. It was observed that water from all samples evaporated and the evaporation rate was high in samples which had cover on the top of the cup. From the table it is evident that evaporation rate of water from A, B, C and D type samples were 1.40±0.001, 1.48±0.001, 1.55±0.001 and 1.33±0.001 (gm/d) respectively when samples were kept without any cover on the cup. Statistically the difference between them were significant (P<0.01). On the other hand evaporation rate from the samples having cover on the top of the cup were 1.05±0.001, 1.20±0.001, 1.28±0.001 and 0.96±0.001 respectively. Statistically the difference between them were significant (P<0.01). From the table it is also found that C type dahi samples of without cover and cover group had the highest evaporation rate (gm/d) and D type samples (plain dahi) of both group had the lowest evaporation rate (gm/d). The evaporation rate of A, B and C type dahi samples of both cover and without cover group might have increased gradually due to incorporation of banana juice. The highest evaporation rate in without cover group was due to the effects of cover. Cover on the top of the cups prevents evaporation and for this reason evaporation rate was low in cover group. Information is very limited on the evaporation rate of dahi during storage in refrigerator. Hence it was not possible to compare the findings of this study with other workers. Broker *et al.* (1974) found that external resistance to vapour removal from product surface is much higher compared to internal resistance to moisture transport to product surface. Uddin and Islam (1985) observed that temperature profoundly influences drying rate and that the temperature, the higher is the rate of drying.

Removal of moisture percentage : Table 2 shows that the removal of moisture percentage from total moisture content of different kinds of dahi samples (A, B, C and D type) of without cover group are 83.20±1.01, 84.61±1.01, 87.95±1.01 and 82.90±1.01 and with cover group are 62.79±0.47, 68.49±0.47, 72.52±0.47 and 59.98±0.47

respectively. Table 2 also indicate that there was a significant differences ($p<0.01$) among the dahi samples of without cover group and with cover group. From the table it is also found that C type dahi sample of with cover and without cover group has the highest removal moisture percentage and D type dahi sample of with cover and without cover group has the lowest removal moisture percentage. The moisture percentage of A, B, and C type dahi samples of with cover and without cover group might have increased gradually due to incorporation of banana juice. On the other hand, Table 2 reveals that the moisture percentage of different kinds of dahi samples (A, B, C and D type) of with cover group are lower than that of dahi samples of without cover group. It was mentioned previously that very limited information has been found on the evaporation rate of dahi during storage in refrigerator. So it was not possible to compare the findings of this study with other workers. But other related works in support of the present findings. Islam (1990) noted that 69% water lost when banana juice was stored at a refrigerated condition. similarly Howkes and Flinks (1978) observed that 72% water was lost when fruit slices were stored in refrigeration.

Conclusion: In both covered and uncovered condition water was removed from all dahi samples. All samples lost weight due to removal of water by evaporation. More water was removed when the samples were put uncovered condition than that of covered condition. So, it is wise to put cover on the top of the container when dahi stored at refrigeration condition.

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Evaluation of Nutritional Status of Recently Hospitalized Patients

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Abstract: Three hundred and fifty five patients of various age groups were assessed for their nutritional status. Among these patients, 65 were children, 166 adult male, and 124 adult female. In children all the anthropometric parameters were lower than the reference values with the exception for age group 6.1 – 10.0 years, whose weight were more than the reference values. Hundred percent mal-nourishment was observed in male adults. All the parameters i.e. weight, upper mid arm circumference (UMAC), triceps-skinfolds (TSF) hemoglobin and blood sugar were lower than the reference values. The data collected for female adults also indicated that the females were hundred percent mal-nourished as well with the exception 51+ years of age whose weights were more than the respective standards or reference values. This study indicates the gravity of the problem of mal-nourishment that exists in the hospitalized patients. Therefore, there is an urgent need for the nutritional assessment/intervention in the hospitalized patients.

Key Words: Malnutrition, nutritional status, hospitalized patients

Introduction

For any productive and useful life, one has to be healthy and well nourished. Nutrition, infact balanced nutrition is most important not only in terms of energy and nutrients but also for the improvement of immunological factors, mental caliber, and physical well-being etc. In order to know health condition and further to know whether or not an individual is well nourished, the assessment of nutritional status is pre-requisite. The aim of the nutritional status is to identify the risk group(s) in a community and to establish a baseline for the evaluation of deficiency(ies) of nutritional regimens and therefore, to facilitate the treatment of the medics or to provide a system of early recognition of the probability of health risk(s) due to nutritional deficiencies. Malnutrition exists any where in the world both in the community and in the hospitalized patients. The incidence of malnutrition in the hospitalized patients is because of many reasons. These are the disease condition itself, the stress of the disease, the stress of the physical/clinical investigations, the fear of facing the doctors and paramedics and of course the feeling of interruption to the privacy of the patient. The increased incidence of malnutrition in the hospitalized patients is associated with greater mortality rates, delayed functional recovery and therefore increased cost of nutritional/medical care both in hospital and at home (Convisky *et al*., 1999). In order to maintain proper nutritional status of a patient, there are some factors, which have to be considered on the day of admission. These includes, specific nutritional screening (clinical/biochemical), dietary investigations and nutritional counseling (Wyszynsky *et al*., 1998). It has been observed that malnutrition/deteriorated nutritional status of a patient is directly related to the stay in hospital. In other words, it means that higher is the degree of malnutrition the longer would be the stay in hospital. This problem is further aggravated due to lack of awareness or negligence of medics or paramedics about the deleterious effects of malnutrition and its clinical outcome. The screening for nutritional risks on the day of admission would enable the nutritionist to identify appropriate degree of intervention for a patient timely and in cost-effective manner (Corrish, 1999; Murphy *et al*., 2000; Corrish and Kennedy, 2000). In some studies, the malnutrition in the hospitalized patients has been reported to be associated with the lack of variety in the food which might limit the consumption of various nutrients (Cabral *et al*., 1998). Most often, the problem of nutritional status goes unrecognized. In critically ill patients it has been shown that patients receiving proper nutritional care shows slow but significant improvement in the nutritional status (Huang *et al*., 2000). Patients at risk for malnutrition have higher costs of recovery from disease (Chima *et al*., 1997). In a study conducted elsewhere, it has been shown that timely nutritional intervention improve the nutritional status of the hospitalized patients (Huang *et al*., 2000). Therefore, early nutritional assessment and appropriate nutrition interventions are required to improve clinical out-come and help to reduce the cost of health care (Hall *et al*., 2000; Wyszynsky *et al*., 1998). Therefore, an increased awareness is required with routine assessment of nutritional status in all hospitalized patients. The present study was conducted on the hospitalized patients in two leading hospitals of the North West Frontier Province (NWFP), Pakistan. The aim of this study was to evaluate the nutritional status of the hospitalized patients and to determine how their nutritional status deviates from their reference variables.

Materials and Methods

Location of the study: The present study was conducted in the two leading hospitals of NWFP, i.e. Khyber Teaching Hospital (KTH), Jamrud Rd, Peshawar and Hayatabad Medical Complex (HMC), Peshawar, Pakistan.

Sample size and selection: Three hundred and fifty five patients of various age groups were assessed for their nutritional status. Among these patients, 65 were children, 166 adult male, and 124 adult female. Since these hospitals are well-established teaching hospitals therefore, the patients are attracted from all of over the province. Therefore, the patients were included in this study without further randomization within the hospitals. The patients belonged to various wards irrespective of the disease with exception that critically ill patients were not included.

Anthropometric measurements: The anthropometric included in this study were weight, height, Head-circumference (in case of children), upper mid arm circumference (in case of adults) and skinfold thickness (in case of adults). The body weight of the children was determined by using the digital beam scale and those of adults with the help of common bathroomscale. Height, head circumference and upper mid arm circumference were determined by using ordinary simple steel measuring tape. The skinfold-thickness was determined by using skinfold caliper.

Biochemical measurements: The various blood tests carried out for the diagnosis of diseases were recorded from the hospital records and compared with reference standards.

Compilation of data: The data collected was compiled and grouped according to the international age grouping system both for children and adult male and females patients. The difference between the actual reading and with the reference standard was determined. Then percent increase or decrease over the reference standard was calculated (WHO, 1983).

Statistical Analysis: The various age groups were coded and the corresponding data was fed to computer for the calculation of mean and standard deviation for the various variables by using a statistical package (MINITAB).

Results

The raw data collected in the various wards of the hospitalized patients was compiled and compared with the international standards. The results are presented in the Tables 1 – 3. In children all the anthropometric parameters were lower than the reference values with the exception for age group 6.1–10.0 years, whose weight were more than the reference values.

The data for the children groups indicates that there was severe malnutrition in the hospitalized children. Similarly, when the data from the clinical test such as hemoglobin (Hb) or immunological factors (not shown in the data) were lower compared with the normal ranges. Most of them were anemic and stunted as well (Table 1 and Fig. 1).

The data grouped for male adolescents and adults and compared with their respective standards as well. Hundred percent mal-nourishment was observed in adolescents and adults. All the parameters i.e. weight, upper mid arm circumference (UMAC), triceps-skinfolds (TSF) hemoglobin and blood sugar were lower than the reference values (Table 2 and Fig. 2). From the hospital records it was noticed that in these patients the immunological factors were not lower (data not shown).

The data collected for female adolescents and adults also indicated that the females were hundred percent mal-nourished as well with the exception 51+ years of age whose weights were more than the respective standards or reference values. All the parameters i.e. upper mid arm circumference (UMAC), triceps-skinfolds (TSF) hemoglobin and blood sugar were lower than the reference values with exception for weight (Table 2 and Fig. 2).

Discussion

This study was conducted in the two main hospitals of NWFP, in order to know whether or not mal-nourishment exits in the hospitalized patients. It is fact that nutritional facilities in the public health care units are not available in this country. However, there are a few private health care units which provide nutritional care and counseling to the patients. In the developed countries, nutrition is recognized as an important factor in maintaining good health and as a preventive measure of diseases. This is the reason that the health care units in the developed countries have established nutrition rehabilitation units (NRUs) and proper nutritional assessment programme in the outpatient departments to provide dietary counseling/service. The root cause of diseases for both communicable and non-communicable is the malnutrition whether it is over-nourishment or under-nourishment. The present study indicates that most of the patients were having lower anthropometric indices (Fig. 1, 2 and 3). The patients either got malnourished in the hospital or they were having history of mal-nourishment. In literature, the observed mal-nutrition in the hospitalized patients has been shown to be because of several reasons. It has been observed that complications are significantly greater for patients who decline nutritionally, regardless of nutritional status at admission, compared with the reference group. This is associated with higher hospital charges and a higher

Table 1: Anthropometric and Biochemical Indices of the Hospitalized Children

Variables	Age Groups (Years)			
	0.5 – 1.0 (N=16)	1.1 – 3.0 (N=17)	3.1 – 6.0 (N=17)	6 .1– 10.0 (N=16)
Weight (kgs	6.0 ± 3.0	6.0 ± 2.0	13.0 ± 3.0	24.0 ± 9.0
Recombinant length (cm)	72.0 ± 23	69 ± 8.0	97 ± 10	-
Head-circumference (cm)	42 ± 0.0	43 ± 3.0	-	-
Hemoglobin (g/dl)	9.0 ± 2.0	9.0 ± 2.0	11.0 ± 3.0	10.0 ±1.0

Table 2: Anthropometric and Biochemical Indices of the Hospitalized Male Adolescents and Adult

Variables	Age Groups (Years)				
	10.1-14.0 (N=33)	14.1-18.0 (N=33)	18.1-24.0 (N=33)	24.1-50.0 (N=33)	50.1+ (N=34)
Weight (kgs)	27.0 ± 3.0	53 ± 6.0	51 ± 7.0	59 ± 8.0	63 ± 10.0
Upper mid arm (cm)	16 ± 0.0	23 ± 3.0	22 ± 2.0	23 ± 3.0	24 ± 3.0
Triceps-skinfold thickness (mm)	5.0 ± 1.0	4.0 ± 3.0	3.0 ± 1.0	5.0 ± 4.0	5.0 ± 3.0
Hemoglobin (g/dl)	9.0 ± 2.0	12.0 ± 1.0	11.0 ± 1.0	11.0 ± 1.0	11.0 ± 1.0
Blood sugar (mg/dl)	80 ± 12	75 ± 14	105 ± 34	102 ± 39	90 ± 24

Table 3: Anthropometric and Biochemical Indices of the Hospitalized Female Adolescents and Adult

Variables	Age Groups (Years)				
	10.1-14.0 (N=26)	14.1-18.0 (N=26)	18.1-24.0 (N=24)	24.1-50.0 (N=24)	50.1+ (N=24)
Weight (kgs)/	42 ±1.0	52 ± 12.0	54 ±16.0	59 ±14.0	51 ± 13.0
Upper mid arm circumference (cm)	19 ± 2.0	24 ± 4.0	23 ± 4.0	24 ± 5.0	23 ± 4.0
Triceps-skinfold thickness (mm)	2.0 ± 0.0	8.0 ±4.0	8.0 ± 6.0	8.0 ± 6.0	7.0 ± 6.0
Hemoglobin (g/dl)	10.0 1.0	11.0 ± 2.0	11.0 ± 0.0	10.0 ± 2.0	11.0 ± 2.0
Blood sugar (mg/dl)	75 ± 0.0	108 ± 46	97 ± 25	96 ± 39	108 ± 66

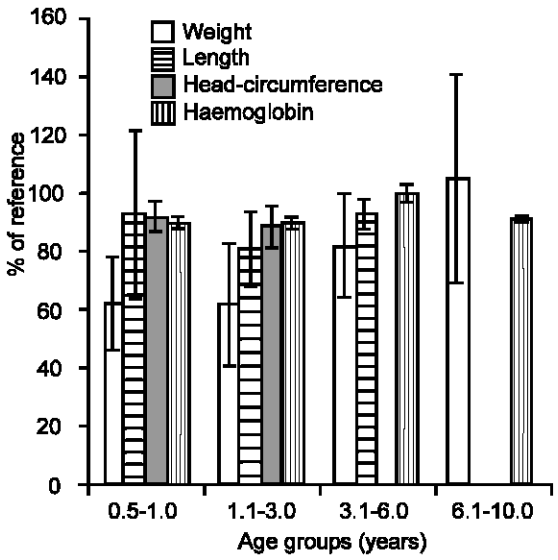

Fig. 1: Anthropometric indices and hemoglobin concentration of the children groups. The values are the mean for various age groups of the reference standard values for the corresponding groups.

likelihood of complications (Braunschweig *et al.*, 2000). It has been suggested that the nutritional assessment upon admission reflects the patient's nutritional and living conditions, current treatment and is predictive of patient's outcome (death or survival) (Gazzotti *et al.*, 2000). In hospital, the catering service systems can have a major impact on the nutritional intake of hospitalized patients (Wilson *et al.*, 2000). It appears that it is crucial to improve the quality of hospital catering, especially to prevent malnutrition (Rigaud *et al.*, 1999). Contributing to the problem of inadequate nutrient intake, patients are frequently ordered to have nothing by mouth and are not fed by any other route (Sullivan, 1999). This is associated with further aggravation of the problem. It has been proposed that evaluation of nutritional status in hospitalized patients is disregarded (particularly in this country) and a simple screening sheet can be used to identify patients in need of further nutritional assessment and treatment (Thorsdottir *et al.*, 1999). In the hospitals, patients show high nutritional instability, with high prevalence of both underweight and overweight. Food habits, demonstrate a lack of variety and a high frequency of food taboos, which might limit the consumption of various nutrients (Cabral *et al.*, 1998). In patients with less severe degrees of illness, the existence of malnutrition leads to a worse outcome than in sicker patients. Malnutrition continues to be a persistent problem in hospitalized patients, which can be readily identified using simple and easily available indices and, furthermore, readily treated (Giner *et al.*, 1996).

The facts reported in the contemporary literature regarding the causes and existence of mal-nourishment

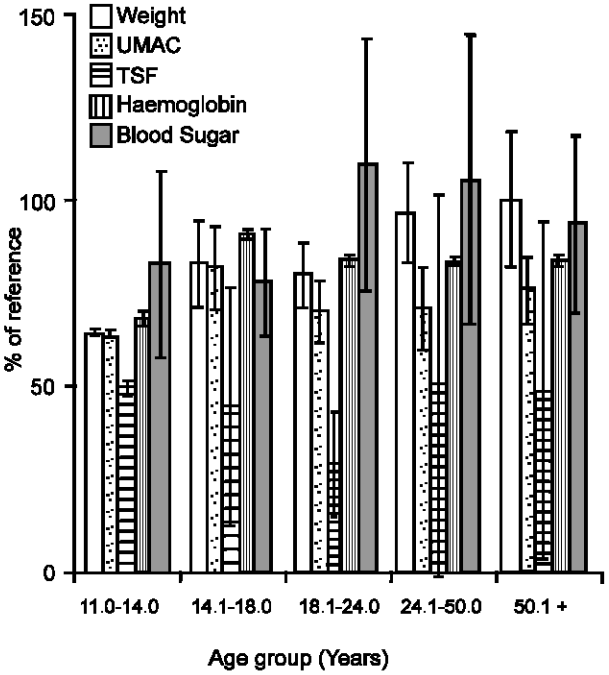

Fig. 2: Anthropometric and biochemical indices of the adult male groups. The values are the mean for various age groups of the reference standard values for the corresponding groups.

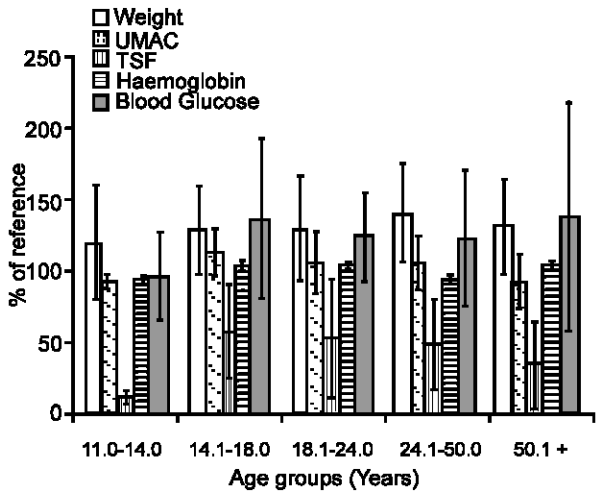

Fig. 3: Anthropometric and biochemical indices of the adult female groups. The values are the mean for various age groups of the reference standard values for the corresponding groups.

in the hospitalized patients can be attributed to the observed mal-nourishment in the patients under report. In medical practice (in this country) meager attention is given to the nutritional intake at the time assessment. In the hospitals for the hospitalized patients, no nutritional history is recorded. This practice has to be individualized in terms of nutritional requirements. This would not only improve out-come but would also reduce the cost of treatment. This study is very limited but it indicates the gravity of the problem of mal-nourishment and there is an urgent need for the nutritional assessment / intervention in the hospitalized patients.

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Prevalence of Diabetes Mellitus and its Relation to Diet and Physical Work in Azad Jammu and Kashmir

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Abstract: The prevalence of diabetes mellitus and its relation to diet and physical work was studied in three selected districts namely Muzaffarabad, Bagh and Poonch of Azad Jammu and Kashmir. A city, a town and a village were selected from each selected district, and hundred households (families) from each city, town and village were randomly selected for the study. A total 900 households were selected for interview. A responsible individual of 40 years or older of each household was interviewed and information about diabetes, occupation and diet were recorded in the questionnaire. The mean prevalence of diabetes mellitus was 0.95% in the selected region of the state. The prevalence of the disease was higher in cities than towns and villages. The disease was slightly higher in males than females. The milk and meat consumption of the residents of the area was lower than the recommended amount. The exercise level/physical work status of majority of the residents was almost equal to heavy exercise level. The data suggest that at present, the prevalence of diabetes is not of great concern in Azad Jammu and Kashmir. The prevalence of the disease is linked with diet and exercise level/physical work. The residents have marginal deficiency of food intake

Key words: Prevalence, diabetes mellitus, diet, physical work

Introduction

Prevalence of diabetes mellitus in various countries has been reported (Zimmet, 1982; Taha *et al*., 1983; Balkau *et al*., 1985; Rosenthal *et al*., 1984; King *et al*., 1984; Omar *et al*., 1985). Obesity, diet and sedentary life style have been named as the major causative factors for the prevalence of the disease. Khan *et al*. (1993b) have reported that 95% of the male and almost all of the female diabetics were over weight by the WHO standard at the onset of the disease. Most of the diabetic individuals used to consume high amount of sugar and fat and were inactive before the onset of the disease. They have suggested that intake of sugar and fat should be minimized and physical labor should be increased to reduce the risk of the disease.

The disease is becoming a major health problem in the elderly population of the world. Precautionary measures must be initiated to control the disease. Reliable data on prevalence and epidemiology of the disease are needed for proper planning. The prevalence and epidemiology of the disease in N.W.F.P and in the Northern Areas of Pakistan have been reported (Khan *et al*., 1993a, 1993b; Khan *et al*., 1999a, 1999b), however, the occurrence of the disease in Azad Jammu and Kashmir is not known. This paper reports the prevalence of the disease and its relationship with food intake and physical work in the mountainous zone of Azad Jammu and Kashmir.

Materials and Methods

Location of the Study: The survey was conducted in the three mountainous districts, namely Muzaffarabad, Bagh and Poonch of Azad Jammu and Kashmir.

Sample Selection for the Study: A city, a town and a villages were selected from each selected district. The district's headquarters of the selected districts were included in the sample. A total of 3 cities, 3 towns and 3 villages (9 locations) were selected. Hundred households (families) from each city, town, and village were randomly selected for the survey. The selected cities, towns and villages are shown in Table 1.

Criteria for Sample Selection: The basic consideration for sample selection was that the selected districts should be located in the mountainous zone of the state and at the same time there should be a maximum variation in the culture and customs of the selected cities, towns and villages. Another point in the selection of sample was that the selected cities, towns and villages should be located at different directions and at sufficient distance from each other and should cover the maximum area of the district. The third point in the selection of sample was that the sample should contain a city, a town and a village for socio-economic, eating habits and pattern and occupation reasons. The selected cities, towns and villages should have transportation facilities.

Procedure for Collection of Data: A responsible individual of age 40 years or above of each household (family) was interviewed and the questionnaire was

filled. The questionnaire was having the information about diabetes, diet and occupation.

Results

The prevalence of diabetes mellitus in the selected cities, towns and villages of the selected districts of Azad Jammu and Kashmir is presented in Table 2. The number of families, the total number of individuals in these families, the number and percent of male and female diabetics and the total percent of the disease in each city, town and village are presented in this table. The highest prevalence of the disease was in the city of Muzaffarabad (2.17%) followed by Bagh city (1.23%) and Rawalakot city (1.09%). The lowest prevalence of the disease was in the village of Nossary (0.46) followed by the villages of Forward Kahuta (0.66%) and Abbaspur (0.84%) respectively. The prevalence of the disease in the towns of Hatian Bala, Dhirkot and Hajira was 0.67, 0.67 and 0.97% respectively.

The prevalence of diabetes mellitus in the selected districts of Azad Jammu and Kashmir is shown in Table 3. The mean prevalence of the disease in the district of Muzaffarabad was 1.05% followed by the district of Poonch (0.98%) and district of Bagh (0.84%). The mean prevalence of the disease in the mountainous districts of Azad Jammu and Kashmir was 0.95%.

The average intake of various food groups per week in the various cities, towns and villages of the selected districts is given in Table 4. The per week intake of milk and milk products in the cities, towns and villages of the different districts varied from 2±1 to 7±4 times per week. The consumption of meat varied from 4±3 to 9±5 times in the various cities, towns and villages of the selected districts. The fruits and vegetables intake was from 6±3 to 13±4 times per week. The cereal intake was from 22±11 to 38±14 times per week.

The mean intake of various food groups per week in the selected districts of Azad Jammu and Kashmir is given in Table 5. The mean per week intake of milk and milk product in the various districts varied from 3±3 to 5±5 times per week. The mean consumption of meat varied from 5±3 to 9±5 times per week in the selected districts. The mean fruits & vegetables intake was from 7±4 to 9±5 times per week. The mean cereal intake was from 31±8 to 33±16 times in the selected districts per week. The mean consumption of milk, meat, fruits & vegetables and cereals was 4±3, 7±4, 8±4 and 32±14 respectively in the mountainous districts of Azad Jammu and Kashmir.

Discussion

Introduction to the Study Area: A brief introduction to the districts selected for the study will help in the interpretation of the data. The selected districts of Azad Jammu and Kashmir were Muzaffarabad, Bagh and Poonch. They are located in the mountainous zone of the state. The racial origin of the residents of these districts are Mongoloid, Arians, Persians, Turks, Arabs and local races. The major occupations are agriculture, grazing of cattle and labor. Some people are in business while others are in government service. Generally, the people are poor and hard working. Due to the mountainous location of the area and due to the occupations of agriculture, live stock and labor, the majority of the residents are classed as people who are doing hard physical work.

The major crops of the selected districts are maize, rice, and kidney beans (Lobia). The staple food is cereals and legumes. Almost all kinds of vegetables are grown. Mushrooms are available as wild crop. A fair amount of honey is produced in the selected districts. Fruits particularly apple, cherry and walnut are abundantly available.

The eating habits and pattern of the selected districts varies from urban to rural areas. The eating routines of urban population is breakfast, mid morning tea, lunch, afternoon tea, dinner and bed time snake. In the breakfast, they take tea with parata (wheat bread prepared with ghee or oil), or tea and bread(wheat or maize) with egg or dal (Pulses). In mid morning tea, they take tea with biscuits, or cake, or samosa or kulcha (a local product prepare from fine wheat flour, fat, egg and sugar). In lunch, they usually take vegetable or vegetable meat curry with bread. Most of the time, butter milk (lassi) becomes a part of lunch. In the afternoon, they take a cup of tea with kulcha. In dinner, they usually take boiled rice with meat or pulses. At bed time eating is not common, however, some people drink milk or take fruit. Consumption of milk and meat groups are poor. Their fruit intake is fair. Their fat consumption may be sufficient. Their physical work level is comparatively low than the people of rural areas. This is because the occupation of urban people are usually business or government/ semi-government jobs. Both the occupations are considered equivalent to light exercise level (Krause and Mahan, 1984).

The eating routines of the rural population is a little different from that of the urban population. Due to their less resources, they cannot afford mid morning, after noon and bed time tea and snake. They usually eat break fast, lunch and dinner. Most of the time, they cannot afford parata or egg. In the breakfast, they drink either tea only or take bread with tea. In the lunch, they usually eat cereal bread with vegetables or butter milk (lassi). In dinner, boiled rice with pulses is the usual routine. They use milk very rarely, perhaps only in tea. Their meat and fruit consumption is nominal. Their fat consumption is low. Because of their rural residence, their occupation is usually agriculture, raising of cattle and labor. They are doing very hard physical work for their survival.

Table 1: Selected Cities, Towns and Villages for the Survey in Azad Jammu and Kashmir

Name of District	Selected Cities, Towns and Villages
Muzaffarabad	Muzaffarabad City, Hatian Bala, Noesary.
Bagh	Bagh city, Dhirkot, Forward Kahuta,.
Poonch	Rawalakot city, Hajira, Abbaspur

Table 2: Prevalence of Diabetes Mellitus in the Selected Cities, Towns and Villages in the Selected Districts of Azad Jammu and Kashmir

Name of City, Village & Valley	Number of families	Number of individuals	Patients		Percentage		Total Percentage
			Male	Female	Male	Female	
Muzaffarabad	100	599	07	06	1.16	1.00	2.17
Hattian Bala (Jehlum Valley)	100	743	02	03	0.26	0.40	0.67
Nossary (Neelum valley)	100	648	01	02	0.15	0.30	0.46
Bagh city	100	728	06	03	0.82	0.41	1.23
Dhirkot	100	737	03	02	0.40	0.27	0.67
Forward Kahuta	100	898	03	03	0.33	0.33	0.66
Rawalakot city	100	728	06	02	0.82	0.27	1.09
Hajira	100	513	02	03	0.38	0.58	0.97
Abbaspur	100	594	03	02	0.50	0.33	0.84

Rationale for Validity of the Data: As one of the goal of the study was to correlate physical work with the incidence of diabetes, so the selection of districts which are located in the mountainous zone of the state, have provided sample of people who are involved in hard physical work. The residents of these districts spend a lot of energy for their normal life activities when they go up and down in their locality. Of course their agriculture and labor profession further add to their physical activity level. In a technical sense, the people residing in the villages and valleys could be classed as people who are involved in strenuous exercise. The criteria that selected cities, villages and valleys should have a maximum variation in the culture and customs, have covered the variation due to culture, customs and eating habits and pattern. The location of the selected cities, villages and valleys at different directions and at sufficient distance from each other have minimized the racial differences as well as the variation due to geographical distance.

As a rationale, the selected sample have heterogeneity, cover the possible ethnic, geographical and socio-economical variations as well as the variations due to culture/customs and food eating habits and pattern.

Prevalence of Diabetes Mellitus: The prevalence of diabetes mellitus in the cities of the mountainous districts of Azad Jammu and Kashmir was higher than the towns and villages of these districts (Table 2). The reason may be the difference in food eating habits/patterns and exercise level/physical work status. In cities, people habitually take more tea and bakery products in addition to their usual food intake. Also, residents of cities are economically well off and eat foods which are high in fat and sugar. People residing in cities, usually take a sweet dish with lunch or dinner. In addition, in cities people are usually involved in business or in government' jobs which keep them less active than others. High intake of fat, sugar and low exercise level are the main causative factors for the incidence of diabetes (Khan and Ahmad, 1994a, 1994b). The prevalence of the disease in the towns and villages was almost equal, indicating that the eating habits/patterns, exercise level/physical work status were similar and therefore , there was no difference in the prevalence of the disease in towns and villages of Azad Jammu and Kashmir.

The prevalence of the disease in the cities of the mountainous districts of Azad Jammu and Kashmir (Table 2) was higher in comparison to the prevalence of the disease in the Northern Areas of Pakistan (Khan *et al.*, 1999a). The highest prevalence of the disease in the Northern Areas was in the city of Gilgit (0.69%), which is almost equal to the lowest prevalence of the disease in the villages and towns of the mountainous zone of Azad Jammu and Kashmir (Table 2). The living conditions like eating habits/ patterns and exercise level/physical work status were almost similar of the two areas. So, the higher prevalence of diabetes in the cities of Azad Jammu and Kashmir could be due to the use of sugar tea. In the cities of Northern Areas, people were very rarely using sugar tea. They are habitual of tea which is prepared with salt. Khan and Ahmad (1994b) have suggested that intake of sugar and fat should be minimized and physical labor should be increased to reduce the risk of the disease.

The prevalence of the disease in Muzaffarabad, Bagh and Rawalakot cities (Table 2) was lower than the prevalence of the disease in the various cities of North

Table 3: Prevalence of Diabetes Mellitus in the Selected Districts of Azad Jammu and Kashmir

Name of City, Village & Valley	Number of families	Number of individuals	Patients		Percentage		Total Percentage
			Male	Female	Male	Female	
Muzaffarabad	300	1990	10	11	0.50	0.55	1.05
Bagh	300	2363	12	08	0.50	0.33	0.84
Poonch	300	1835	11	07	0.59	0.38	0.98
Total of the Selected	900	6188	33	26	0.53	0.42	0.95

Table 4: Mean Intake of Food in the Selected Cities, Towns and Villages of the Selected Districts of Azad Jammu and Kashmir

Name of City, Village & Valley	Number of individuals	Food Groups[1] eaten per week			
		Milk[2]	Meat[3]	Fruits and Vegetables[4]	Cereals[5]
Muzaffarabad city	100	7 ± 4	9 ± 4	13 ± 4	29 ± 6
Hattian Bala (Jehlum Valley)	100	5 ± 4	9 ± 5	9 ± 1	34 ± 9
Nossary (Neelum Valley)	100	3 ± 3	8 ± 4	10 ± 3	28 ± 6
Bagh city	100	2 ± 1	9 ± 3	9 ± 2	29 ± 3
Dhirkot	100	4 ± 3	6 ± 4	6 ± 5	32 ±14
Forward Kahuta	100	4 ± 3	7 ± 3	7 ± 3	38 ±14
Rawalakot city	100	5 ± 4	6 ± 3	8 ± 4	25 ±12
Hajira	100	3 ± 1	4 ± 3	6 ± 3	22 ±11
Abbaspur	100	4 ± 3	6 ± 3	7 ± 4	38 ±13

1 The figures in the column of each food group indicate the mean and standard deviation (N=100) of each city, town and village. The number is not the serving size in its usual meaning but it is the number of times they ate the particular food group per week.

2 Indicates glasses of milk or butter milk (lassi) or bowl of yogurt; almost equal to the usual meaning of serving size in milk.

3 Indicates one to two pieces of beef in a plate of mixed beef vegetable curry or very rare a piece of chicken in a plate of curry, or a plate of pulses; less than the usual meaning of serving size in meat. The vegetables quantity was almost equal to the usual meaning of serving size in vegetables.

4 Indicates fruits like 3-4 number of fresh apricot or cherry or one apple or a glass of fruit juice or 5-6 number of walnut etc. and a plate of vegetable like potato or spinach etc.; almost equal to the usual meaning of serving size in fruit

5 Indicates wheat bread, corn bread, biscuits, kulcha and rice; more than the usual meaning of serving size in cereals

West Frontier Province (NWFP) of Pakistan. The prevalence of the disease in Peshawar and Mardan cities was 3.57 and 3.53% respectively (Khan *et al.*, 1993a). The lower prevalence of the disease in the selected cities of Azad Jammu and Kashmir in comparison to cities of NWFP was perhaps due to high exercise level/ physical work status and less urbanized nature of the cities of Azad Jammu and Kashmir. Also obesity, a risk factor for the disease was not prevailing in Azad Jammu and Kashmir (unpublished data of the author). The absence of obesity may also be a cause for the lower prevalence of the disease in Azad Jammu and Kashmir . Khan and Ahmad (1994a) have reported that obesity is the major causing factor for the prevalence of diabetes mellitus.

The data in Table 2 clearly indicate that those individuals, who were residing in more urbanized cities, were having higher prevalence of the disease in comparison to those who were residing in more rural towns and villages. This was perhaps due to more tea and sugar intake in cities as compared to towns and villages. Low exercise level/ physical work status in cities due to their occupation as business and government jobs may also be a reason for the high prevalence of the disease. High exercise level and physical training reduces the incidence of diabetes mellitus (Khan and Ahmad, 1994a; Mohs *et al.*, 1985).

The prevalence of diabetes mellitus in the selected districts of Azad Jammu and Kashmir is shown in Table 3. The prevalence of the disease in the district of Muzaffarabad was 1.05% followed by the district of Poonch (0.98%) and district of Bagh (0.84%). There was not much difference in the mean prevalence of the disease in the selected districts of Azad Jammu and Kashmir. The little difference might be due to the urbanized nature of the various districts.

The overall prevalence of the disease in the mountainous region of Azad Jammu and Kashmir was 0.95% (Table 3), which was much higher than the prevalence of the disease (0.17%) in the Northern Areas of Pakistan (Khan *et al.*, 1999a). However, the prevalence of the disease in the selected region of Azad

Table 5: Mean Intake of Food in the Selected Districts of Azad Jammu and Kashmir

Name of City, Village & Valley	Number of individuals	Food Groups[1] eaten per week			
		Milk[2]	Meat[3]	Fruits and Vegetables[4]	Cereals[5]
Muzaffarabad	300	5 ± 5	9 ± 5	9 ± 5	31 ± 8
Bagh	300	3 ± 3	7 ± 3	8 ± 4	33 ± 16
Poonch	300	4 ± 3	5 ± 3	7 ± 4	32 ± 17
Mean Values	900	4 ± 3	7 ± 4	8 ± 4	32±14

1 The figures in the column of each food group indicate the mean and standard deviation (N=300) of each district. The last row indicates the mean values of all the districts. The number is not the serving size in its usual meaning but it is the number of times they ate the particular food group per week.

2 Indicates glasses of milk or butter milk (lassi) or bowl of yogurt; almost equal to the usual meaning of serving size in milk.

3 Indicates one to two pieces of beef in a plate of mixed beef vegetable curry or very rare a piece of chicken in a plate of curry, or a plate of pulses; less than the usual meaning of serving size in meat. The vegetables quantity was almost equal to the usual meaning of serving size in vegetables.

4 Indicates fruits like 3-4 number of fresh apricot or cherry or one apple or a glass of fruit juice or 5-6 number of walnut etc. and a plate of vegetable like potato or spinach etc.; almost equal to the usual meaning of serving size in fruit

5 Indicates wheat bread, corn bread, biscuits, kulcha and rice; more than the usual meaning of serving size in cereals

Jammu and Kashmir was much lower than the prevalence of the disease (1.49%) in NWFP (Khan *et al.*, 1993a). High sugar intake, sedentary life style and occupation play a direct role in the development of diabetes mellitus (Khan and Ahmad, 1994a; b; Mohs *et al.*, 1985; Khan *et al.,* 1999b). The variation in the prevalence of the disease in Northern Areas, NWFP, Pakistan and Azad Jammu and Kashmir are understandable, because the residents of Northern Areas have more tough life and they use salt instead of sugar in tea and other dishes. The residents of NWFP use more sugar and sweets in their daily routine. They also have low exercise level/physical work status, because the major population of NWFP is living in plane areas. In Azad Jammu and Kashmir, though people are using sugar in tea and other dishes, yet they are at higher exercise level/physical work status because of their residence in the hilly areas and because of their occupations which are usually labor or agriculture.

Prevalence of Diabetes Mellitus in Relation to Diet and Physical Work: The mean intake of various food groups per week in the various cities, towns and villages of the selected districts of Azad Jammu and Kashmir is given in Table 4 . It should be noted that the figures of intake of each food group were not the serving size in its usual meaning. Actually it was the number of time they ate that particular food group at a particular time. For example, intake of tea in this study stands for one cup at any particular time. Similarly, intake of milk or butter milk (lassi) indicates a glass of milk or butter milk (lassi) at any particular time.

From Table 4, it is clear that consumption of milk and meat was sufficiently low. The per week intake of milk and milk product in the various cities, towns and villages of the various districts varied from 2±1 to 7±4 per week which is very low in comparison to the recommended intake of 14 per week (Whitney and Hamilton, 1981). The consumption of meat varied from 4±3 to 9±5 in the various cities, towns and villages of the selected districts. The consumption of pulses were included in the meat group. But still the meat consumption was low. The recommended intake of meat for adults is 2 servings per day i.e. 14 servings per week. (Whitney and Hamilton,1981). The low intake of milk and meat groups may produce protein, calcium, iron and other nutrients deficiency as the above food groups are rich in these nutrients. The general observation of health of many individuals provided a clue for marginal nutrients deficiency. Apparently they look healthy, perhaps they have adapted themselves to the minimum requirements of these nutrients. Khan *et al.* (1993c) have reported that animals/human can be adapted to lower level of nutrients requirement.

The fruit intake might be a little less than the recommended intake, but the vegetables intake was sufficient or even more than the recommended level, as the amount of vegetables taken at one time was more in quantity than the known serving size of fruits and vegetables groups.

The cereal intake was from 22±14 to 38±14 times per week. The recommended intake level is 4 servings per day i.e. 28 servings per week (Whitney and Hamilton, 1981). So the cereal intake of the residents of the selected region was enough or might be a little more as the amount taken was more than the usual serving size. Generally, the residents seems to be marginal deficient in protein, calcium and iron. The residents may be getting enough or little low energy for normal requirements, but as they are doing hard physical work for which extra energy is needed, so the residents of the selected region may be on small negative energy balance. Khan *et al.* (1999b) have reported that the intake of food on average basis is low and imbalanced in the Northern Areas of Pakistan. They have argued that the low prevalence of diabetes in the Northern Areas is due to absence of obesity, diet and physical exercise.

Except for salty tea in the Northern Areas, the other conditions of the Northern Areas and the selected region of Azad Jammu and Kashmir were similar. With the present eating habits and intake, it is expected that the prevalence of diabetes will be low in the selected region of the state. This statement is supported by the data shown in Tables 2 and 3, where the prevalence of diabetes was much lower than the prevalence of diabetes in the NWFP, Pakistan (Khan and Ahmad, 1994a) and other countries (Zimmet, 1982; Taha *et al.*, 1983; Balkau *et al.*, 1985; Rosenthal *et al.*, 1984; King *et al.*, 1984; Omar *et al.*, 1985).

The mean intake of various food groups per week in the selected districts of Azad Jammu and Kashmir is given in Table 5. The mean consumption of milk, meat, fruits & vegetables and cereals was 4±3, 7±4, 8±4 and 32±14 times per week, respectively, in the selected districts of Azad Jammu and Kashmir. This indicated that the mean consumption of milk and meat in Azad Jammu and Kashmir was lower than the recommended intake level of these groups (Whitney and Hamilton, 1981). As already discussed, the low intake of these groups may produce important nutrients deficiency. Apparently, the residents of the area look healthy but this may be due to the adaptation behavior of animals and humans where they can adapt to lower nutrients requirement in nutritional stress conditions. Khan *et al.* (1993c) have reported that animals/human can be adapted to lower level of nutrients requirement in nutritional deficiency states.

Dietary intake is an important factor contributing to the incidence of the diabetes mellitus. Tables 4 and 5 show the over all picture of food intake in Azad Jammu and Kashmir. The diet of the area on average basis is imbalance. Diabetes mellitus is a disease where the body become unable to handle the glucose which is taken in foods. It has been reported that imbalance diet which is high in simple sugar and fat causes diabetes mellitus in the later stages of life (Mohs *et al.*, 1985; Khan *et al.*, 1993b, 1999b; Khan and Ahmad, 1994b). Exercise level/physical work status also influence the prevalence of diabetes of mellitus. The importance of physical training and exercise level have been reported by many researchers (Franz, 1987; Lingarde *et al.*, 1983; Chandler, 1977).

In the light of this study, the prevalence of diabetes mellitus is not of major concern In the Mountainous zone of Azad Jammu and Kashmir. High sugar intake causes the disease and high exercise level/physical work reduces the disease. The residents are advised to use less sugar in their daily life and involve themselves in more physical work. Sugar tea should be limited to a cup or tow per day. The individuals who are involved in business and government service should make a routine for daily exercise.

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Surveillance on Artificial Colours in Different Ready to Eat Foods

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Abstract: Different types of ready to eat foods were analyzed for the detection and estimation of the added synthetic food colours. A total of 72 different samples of sweet meats and confectioneries were randomly collected from large and small shops of Rawalpindi Cantt. Quantities of the permitted colouring matter among the tested samples were found within the range of 18-220 ppm. 47.56% of the samples contained non-permitted food colours. Incidences of the use of non-permitted food colours and colours above permissible limits were higher in case of the unorganized food makers.

Key words: Sweet meats, natural food colours, synthetic food colours

Introduction

From the surveillance data published in different countries it is evident that food intoxication rather than abating are on the increase. It is observed that coloured products are the major source of food intoxication and surveys conducted to determine the presence of non-permitted food colours in different food products. It is instinct phenomenon that human are always attracted to foods and drinks bearing pleasant colours. Adding attractive colours can definitely enhance the appetizing value of food and drink and in order to make the food attractive for customers, most manufacturers use different colours in their bakery products such as cakes biscuits and pastries. Similarly colours are often used in the manufacture of soft drinks various kinds of toffees, ice-creams, jams and jellies etc, by both street vendors and large manufacturers. Even house wives use dyes to colour rice and other dishes, mainly to give them a more appetizing look.

Many of these dyes were originally derived from coal tar and were commonly called coal-tar dyes, they contain the azo group. Colour chemicals are by definition active chemicals hence require greater care than bland additive such as emulsifiers.

Natural food colours are extracted and isolated from different Plants and animals as they have no harmful effect so they can be used in any food in any amount. These colour are less stable, less bright and not uniform but they are very expensive moreover it is also difficult to find the exact shade required for different food products.

According to the Pure Food rules of 1965 eighteen synthetic food colours and five natural food colours are permitted food colours in Pakistan. The synthetic food colours are Food Blue, Food Violet, Food Green Red and Food Black. The use of Black and Brown dyes is completely banned in the developed countries as they contain harmful ingredients. The maximum limit of permissible colour to be added in any food shall be 0.1 gram per Kg of food as consumed.

Even permitted artificial colours, if consumed indiscriminately are not safe. Citizens are still forced to consume adulterer edibles. The greedy elements mix lead chromate in turmeric (causing anemia, blindness and other disabilities), inedible colours in beverage (cause liver disorders and cancer). Higher incidence of gastrointestinal diseases cholera, enteric fever, tuberculosis etc. can also be traced to food infected with the causative organisms of these diseases. (Davis *et al.*,1964; Maurer *et al.*, 1980). Medicines, however, costly and effective can never replace good food. In actual practice, other non-permitted synthetic dyes like auramine, methanol yellow, lead chromate, rohdamine sudan-3 and 4, orange-1 and malachite green, which pose serious health hazards, as they are mutagenic and potential carcinogens, are being used as food colours in the market.

Different studies elsewhere revealed that analysis of various food products with respect to the added colours showed the concentrations of food colours in different food products ranges from 15-20 mg/kg, which was within the minimum permissible limit. It was found that a few foods manufactured by unorganized private sector and small vendors did contain colours in higher concentration than permitted range (Biswas *et al.*, 1994). Most of the food colours tested in conventional toxicity experiment showed toxic effect at a very high level of in take that were not normally encountered. Human studies indicated that food colours could induce a wide range of allergic reactions only in sensitive individuals (Babu and Shenolikar, 1995).

Keeping in view the importance of the subject present research is carried out to find out the percentage of sweet samples which contain non-permitted colours and to identify the source of colouring matter present in various sweets whether they are from the permitted list or not.

Materials and Methods

Sampling protocol: Seventy three samples of different

sweets containing colours and confectionaries were collected at random from different shops of Rawalpindi Cantt, from July to September 2001. They were analyzed to check the colours used in them whether they were permitted or not and to check the intensity of these colours. First of all the colours were extracted from the samples and then paper chromatographic technique was used for analysis.

Method for Colour Extraction: Synthetic dyes were isolated according to method described by Woodman (1941) For liquid foods they were used directly, while solid and semi foods were dissolved in water before isolation of colours.

About 25-50 mg of sample or its solution was added in the beaker, few drops of glacial acetic acid and white animal wool was added in the beaker then the contents of the beaker were boiled for a few minutes. The wool took any synthetic dye if present. The wool was removed from the beaker, washed under running tap water and finally with distilled water. The colour from the dyed wool was recovered by boiling it in a dilute ammonium hydroxide solution. The wool was discarded and the colour solution was evaporated on water bath after the removal of ammonia solution the colour was dissolved in a 2-3 ml of distilled water and evaporated again on the water bath. The dry colour then obtained was dissolved in few drops of water and stored in a stoppered glass bottle for further analysis.

Identification of Extracted Dyes : The extracted colours were identified by using Paper Chromatography according to the procedure prescribed by Biswas *et al.,* 1994.

Results and Discussion

There is a great need to create awareness at different levels about the toxic effects of non-permitted colours. Different studies elsewhere reported that these food colours might cause health hazards affecting kidneys and causing allergies, gastrointestinal and cancer problems. This study has been carried out for the products manufactured by organized and unorganized sectors and the samples were collected randomly from the different shops of Rawalpindi Cantt over a period of two months. The samples included food items i.e. sweet meats and confectioneries. The standard method was followed for the detection and estimation of colours in samples.

Table 1 Shows that 73 samples were examined, results of these samples revealed that 58.5% of the samples contained permitted food colours within the prescribed limit of prevention of Food Adulteration where as 41.1% samples contain permitted food colours above prescribed limits (PFA, 1992), while 46.57% of the samples contain non-permitted colours. This variation among different samples may be due to their different chemical compositions of food and at the same times their manufacturing processes. Organized companies contain the samples with the food colour with in PFA limit while the samples from the small shops contain colour above the permissible level. In the case of confectioneries, from small shops and sweets they were found to contain undesirable quantities of food colours. Amaranth, Ponceaw 4R, Sunset yellow, Tartrazine, Blue F.C.F, carmosine. Rohdamine B are identified permitted colours while orange 11, Metanil yellow, Congo Red, Blue V.R.S are the identified non-permitted food colours in the examined samples. An unidentified chocolate brown colour is found in few hard boil confectioneries. Survey of this study also revealed that they are also using the colours of the textile.

The health hazard due to consumption of food colours has also been reported by FAO/WHO in 1994. Anaphylactic reactions after consuming natural colours like Annatto were exhibited by certain individuals. (Nish *et al.*, 1991). Food anaphylaxis following ingestion of carmine, a natural dye extracted from the cochnineal insects was reported in women at a dose of 1mg/ kg body wt although the ADI is 0 – 5. mg / kg body weight (Beardowin and Kanny, 1995).

Tartrazine is also a permitted yellow colour. It was frequently found in almost all kinds of examined samples specially in sugar confectioneries. It has been reported to be associated with irritability, restlessness and sleep disturbance in a topic or hypertensive children aged between two and fourteen years (Rowe and Rowe, 1994). A typical case of anaphylactoid purpura associated with tartrazine has been reported (Wuthrich, 1993). Other permitted food colours such as Amaranth, sunsets yellow and Ponceau 4R have also been implicated in adverse reactions in patients with chronic urticaria (Lockey, 1977).

Metanil yellow, the frequently used non-permitted food colour was found to cause toxic methaemoglobinaemia in adult human males 2-4 hours after the consumption of rice coloured with it (Sachdeva *et al.*, 1992). It is also reported to cause cyanosis (Chandro and Nagaraja, 1987). It was observed that RF values of isolated colours from different samples were fourteen red, fourteen blue, eight green, fifteen yellow green in twenty two orange.

In annual report of National Institute of Nutrition 1993, it has been reported that there was a disease outbreak involving 40 school children in Hyderabad. Major symptoms in the affected children were glossitis and burning sensation of the tongue while eating food. The cause was traced to the aniseed mixed with ponceau 4R which were consumed by them. Though ponceau 4R is a permitted food colour, under the PFA Act, it is not permitted to be used in aniseed. In Pakistan no rules and regulations are followed by the manufacturers because government has not implemented any rule

Table 1: Summary of Survey of Collected Different Varieties of Food for Colour Estimation

Category	Sample Analyzed	Samples Containing non-permitted food colours	Samples containing permitted food colours	
			Within PFA Limit	Above PFA Limit
Gulab Jaman	6	3	2	1
Barfi	6	3	2	1
Cham Cham	5	2	1	1
Patisa	5	2	1	1
Ras Gulla	6	2	2	0
Ladoos	5	3	1	2
Kala Kand	5	3	2	1
Jalaebi	5	3	1	2
Toffees	5	2	2	0
Candyes	5	1	1	0
Bubble	5	2	1	1
Chocolate	5	2	1	1
Lolly Pop	5	1	1	0
Street Vendors	5	5	2	3
Total No.	73	34	20	14
Percentage		46.57%	58.5%	41.1%

about the non-permitted food colours. Therefore as a result of this, there is no awareness among the people about health hazards of use of non-permitted food colours.

Conclusion: The result of this preliminary study revealed the frequency of occurrence of permitted colouring matter as well as indiscriminate use of non-permitted colours in some ready to eat foods available in and around Saddar Bazar. The observations indicate that food colours, whether natural or synthetic can induce wide range of adverse reactions in sensitive individuals and these non-permitted colours may be responsible or considered as carcinogenic in nature. Therefore a systematic approach to evaluate the frequency of the occurrence of toxic and non-permitted colours and permitted colours for the estimation will be carried out with in the country. At the same time we must also enforce certain rules or laws to prevent ill effects of using of non-permitted colours as well as permitted colours above permissible level.

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Quality Evaluation of Market Yoghurt /Dahi

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Abstract: This study was planned to evaluate and compare the quality of market yoghurt and dahi. Different samples of plant made yoghurt and dahi available in local markets of Islamabad and Rawalpindi were randomly collected and analyzed for physico- chemical, microbiological and organoleptic properties. Physico-chemical analysis revealed that plant made yoghurt samples were consistent and hardly showed any variation as compared to dahi. Microbiological examination showed that total viable count in yoghurt brands was less than dahi. The coliform count was nil or ignorable in yoghurt brands but dahi contained large number of coliform bacteria. Organoleptically, plant made yoghurt was found more suitable as compared to dahi.

Key words: Physico-chemical, Microbial quality, yoghurt, dahi

Introduction

Yoghurt is perhaps the oldest fermented milk product known and consumed by large segments of our population either as a part of diet or as a refreshing beverage. It is nutritiously balanced food containing almost all the nutrients present in milk but in a more assimilable form. It is believed that yoghurt has valuable therapeutic properties and helps curing gastrointestinal disorders (Athar, 1986).

Yoghurt is derived from Turkish Word "Jugurt" reserved for any fermented food with acidic taste. It involves the use of specific symbiotic/mixed culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Kon, 1959). However, when culture is undefined, the product is called as dahi and contains mixture of various strains of lactic acid bacteria. Thus the quality of dahi may vary with the type of starter culture used (Masud *et al.,* 1991). Yoghurt has assumed different forms in the market. In Pakistan, two main types are set and stirred yoghurt. Of all the varieties set yoghurt with a rather firm body is most common.

The quality of yoghurt/dahi in local market varies from shop to shop as there is no well described standard for these fermented products. However, meanwhile people are becoming more conscious about the quality of these fermented products. Poor quality milk, unhygienic practices associated with the process involved and the use of "wild type" of starter culture give rise to poor grade dahi having six to twelve hours shelf-life only. The alfresco-vending, loose unpacked availability and hence contamination there upon further deteriorates keeping quality (Aziz, 1985). So to ensure the proper quality of yoghurt/dahi, there should be a complete check on the yoghurt and dahi sold in local markets. In Pakistan, dahi is prepared without any care of quality control and hygienic conditions and contains lot of contaminants, which may be health hazards. At the same time various means and methods are adopted in its preparation so there can be seen a lot of variation among the quality of this product.

A practical approach towards the quality of yoghurt/dahi is to evaluate the different samples of yoghurt and dahi sold in local markets. Research in the field of quality evaluation of market yoghurt/dahi is the basic need to create awareness among common people about the existing situation and protect the consumers' health and rights. Therefore, this study was designed to evaluate market yoghurt and dahi for physico-chemical, microbiological and organoleptic properties.

Materials and Methods

The research work was conducted at the Dairy Technology Laboratory, Animal Sciences Institute, National Agricultural Research Center, Islamabad.

Collection of samples: Twenty five samples of dahi and ten samples each of various brands of yoghurt viz., A, B, C totaling thirty samples were collected randomly from local market under sterilized conditions during the month of October, 2001 to March, 2002 i.e., winter season and analyzed for physico-chemical, microbiological and organoleptic characteristics.

Product analysis

Physico-chemical Analysis

Fat: Fat was determined by Gerber method as described by Pearson (1976).

Total Solids: Total solids were determined by AOAC method No.925.23 (1990)

pH: pH was measured by Electronic digital type Hana pH meter No. H_1 8416 according to method No. 981.12 of AOAC (1990).

Total Titratable Acidity: Acidity was measured by AOAC method No.947.05 (1990).

Volatile Acidity: Total volatile acidity was measured according to Winton and Winton (1945) with following modifications. A 10 gm sample and 200 ml distilled water was added into distillation flask and volatile acid compounds were distilled off at very low heat into conical flask. Then it was titrated against 0.1N NaOH solution and total volatile acidity expressed as ml percent.

Viscosity: Viscosity of the yoghurt/dahi samples was measured in terms of relative and apparent viscosity i.e. consistency using an improvised consistometer, as described by the Cole- Parmer (2000). This meter works on the principle of measuring resistance to flow under gravitational force. A known quantity (15 grams) of uniformly and consistently stirred sample was allowed to flow under its own weight for 15 seconds. The distance covered by moving edge of each sample was noted and compared with the standard yoghurt (prepared in the laboratory by addition of 0.5 percent gelatin and 2 percent starter culture in pasteurized milk and then incubation at 42 °C). The ratio between the distance traveled by the sample and the standard yoghurt was reciprocated and then converted into percentage of viscosity by multiplying with 100.

Syneresis: Syneresis was measured according to Peri *et al.* (1985).

Microbiological Analysis: The microbiological analysis of samples was carried out for total viable count and coliform count by method as described by Resubal (1977).

Organoleptic Evaluation: All the samples were evaluated for sensory characteristics and overall acceptability by a panel of judges selected from Dairy Technology Lab., NARC, Islamabad using nine point hedonic scale as described by Larmond (1977).

Statistical Analysis: The data obtained was statistically analyzed according to Steel and Torrie (1980).

Results and Discussion

The research was carried out to study the quality of dahi and plant produced various yoghurt brands available in the local markets of twin city Islamabad/ Rawalpindi. Twenty five samples of dahi and ten samples each of various brands of yoghurt viz., A, B and C totaling thirty samples were collected randomly from the local markets under stringent hygienic conditions during the month of October, 2001 to March, 2002 i.e. winter season and were analyzed for physico-chemical, microbiological and organoleptic characteristics.

Physico-chemical analysis

Fat: The fat content of dahi and three brands of yoghurt i.e. A, B and C are shown in Table 1. The average fat content of dahi was 3.75 with a standard deviation of 0.76. The average fat content of brand A yoghurt was 3.5 with standard deviation of 0.02. These results are in accordance with the findings of Athar (1986) who reported 3.5 percent fat in typical plain yoghurt. The average fat content of brand B and C was 2.99 and 2.94 with standard deviation of 0.02 and 0.04, respectively. The results observed confirmed the findings of Hofi *et al.* (1978).

There was hardly any variation in fat content of different samples of plant made yoghurt probably because of good manufacturing practices i.e. hygiene, quality control and standardization of raw milk. But in case of dahi making the starting material i.e. milk is used without quality control or standardization resulting in compositional variation from sample to sample similarly as milk composition vary from day to day or batch to batch.

Total Solids: The total solids content of dahi and three brands of yoghurt i.e. A,B and C are shown in Table 1. The average total solids content of dahi was 13.38 with standard deviation of 1.34. These results are in line with findings of Hofi *et al.* (1978). The average total solids content of A, B and C was 14.96, 12.93 and 15.73 with standard deviation of 0.02, 0.05 and 0.18, respectively. The results are in accordance with the findings of Athar (1986). These results are totally different from those reported by Sarkar *et al.* (1996).

There was hardly any variation in total solids of different samples of plant made yoghurt brands most probably because of standardization of raw milk and quality control measures taken to ensure consistency of end product. But in case of dahi milk is used without subjecting to standardization leading to much variation as observed in total solids content of market dahi samples.

Solid- Not-Fat (SNF): The SNF content of dahi and three brands of yoghurt i.e. A, B and C are shown in Table 1. The average SNF content of dahi was 9.64 with standard deviation of 1.19. Where as the average SNF content of yoghurt A, B and C was 11.47, 9.94 and 12.78 with standard deviation of 0.01, 0.04 and 0.14, respectively. These results are in agreement with the findings of Richter *et al.* (1980).

There was found no significant variation in SNF content of different samples of plant made yoghurt because raw milk is standardized to a fixed SNF content in order to ensure consistency of end product. But in case of dahi raw milk is used without subjecting to standardization. Hence more variation was observed in SNF content of dahi samples.

Table 1: Physico-chemical analysis (Mean±SD) of dahi and yoghurt samples collected from local markets of Rawalpindi/Islamabad

Product	Fat	Total Solids%	SNF%	PH	Titratable Acidity%	Volatile Acidity Ml%	Viscosity %	Syneresis %
Dahi	3.75 ± 0.76	13.38 ± 1.34	9.64 ± 1.19	4.54 ± 0.24	1.16 ± 0.32	0.83 ± 0.23	46.04 ± 31.90	36.8 ± 8.84
Yoghurt								
A	3.50 ± 0.02	14.96 ± 0.02	11.47 ± 0.01	4.55 ± 0.02	0.89 ± 0.02	0.62 ± 0.02	91.9 ± 4.84	25.4 ± 0.81
B	2.99 ± 0.02	12.93 ± 0.05	9.94 ± 0.04	4.57 ± 0.03	0.87 ± 0.04	0.61 ± 0.03	59.0 ± 2.98	29.64 ± 0.52
C	2.94 ± 0.04	15.73 ± 0.18	12.78 ± 0.14	4.35 ± 0.03	1.13 ± 0.05	0.85 ± 0.04	116.2 ± 11.27	22.8 ± 0.92

Table 2: Microbiological analysis (Mean±SD) of dahi and yoghurt samples collected from local markets of Rawalpindi/Islamabad

Product	Total Viable Count *(10^7)	Coliform Count *(10^3)
Dahi	7.34 ± 1.57	4.39 ± 1.08
Yoghurt		
A	5.61 ± 0.06	0
B	3.31 ± 0.09	0.71 ± 0.96
C	6.34 ± 0.04	3.39 ± 0.5

pH: The pH of dahi and three brands of yoghurt i.e., A, B and C are summarized in Table 1. The average pH of dahi was 4.54 with standard deviation of 0.34. Where as the mean pH values of A, B and C brand were 4.55, 4.57 and 4.35 with standard deviation of 0.02, 0.03 and 0.03, respectively. These results are in line with the findings of Salji *et al.* (1985) and Varnam and Sutherland (1994) There was found no significant variation in pH of different samples of plant made yoghurt as compared to dahi because yoghurt is incubated for specific time and temperature to attain desired pH, which is about 4.6 i.e. isoelectric point of casein. In case of dahi proper fermentation conditions are not fully controlled, hence a large variation of pH in the end product is obvious. A decrease in pH with time interval of storage is naturally expected (Ahmad, 1994).

Total Titratable Acidity: The total titratable acidity of dahi and three brands of yoghurt i.e. A, B and C are shown in Table 1. The average acidity of dahi was 1.16 with standard deviation of 0.32. The average acidity of three brands of yoghurt A, B and C were 0.89, 0.87 and 1.13 with standard deviation of 0.02, 0.04 and 0.05, respectively. These results are in accordance with the findings of Davis and Mclachlan (1974).

There was less variation in acidity of different samples of plant made yoghurt as compared to dahi due to controlled incubation and postproduction handling and controlled storage at 4 °C, so acidity remains same throughout all seasons. While in case of dahi uncontrolled incubation and postproduction handling and storage cause increase in acidity during summer and subsequent decrease during winter season.

Volatile Acidity: The volatile acidity of dahi and three brands of yoghurt i.e. A, B and C are shown in Table 1. The average volatile acidity of dahi was 0.83 with standard deviation of 0.23. Where as the mean values of volatile acidity of yoghurt A, B and C were 0.62, 0.61 and 0.85 with standard deviation of 0.02, 0.03 and 0.04, respectively. These results are not agreement with Hofi *et al.* (1978).

There was found no significant variation in volatile acidity of different samples of plant made yoghurt as compared to dahi because defined starter culture is used and controlled postproduction storage prevents the production of excessive acidity. As acidity is strongly correlated with volatile acidity so production of excessive volatile acidity is also inhibited. In case of dahi undefined starter culture containing different species of lactogenic bacteria including *E. coli* results in the production of more acidity and ultimately more volatile acidity. Also due to unchecked post production storage large variation in volatile acidity is naturally there.

Viscosity: The viscosity of dahi and three brands of yoghurt i.e. A, B and C are shown in Table 1. The average viscosity of dahi was 46.04 with standard deviation of 31.90. The average viscosity of yoghurt A, B and C was 91.9, 59 and 116.2 with standard deviation of 4.84, 2.98 and 11.27, respectively.

There was less variation in viscosity of different samples of plant made yoghurt as compared to dahi because stabilizer is usually used at the rate of about 0.5 percent; hence the yoghurt was consistently viscous. Large variation in viscosity of dahi samples may be due to fluctuation in the quality of raw material i.e. milk and non-adherence to good manufacturing practices (GMP).

Syneresis: The syneresis of dahi and three brands of yoghurt i.e. A, B and C is shown in Table 1. The average syneresis of dahi was 36.8 with standard deviation of 8.84. Where as the mean values of syneresis of yoghurt A, B and C was 25.4, 29.64 and 22.8 with standard deviation of 0.81, 0.52 and 0.92, respectively.

There was less variation in syneresis of different samples of plant made yoghurt as compared to dahi due to presence of stabilizer used in manufacture of yoghurt, which binds and holds water from escaping out. Dahi contains no stabilizer so syneresis is more pronounced but some samples of dahi gave little syneresis comparable to yoghurt. It was due to presence of more total solids. Secondly homogenization in case of

Table 3: Organoleptic evaluation (Mean±SD) of dahi and yoghurt samples collected from local markets of Rawalpindi/Islamabad.

Product	Colour	Taste	Flavour	Overall Acceptability
Dahi	6.78 ± 1.40	4.57 ± 1.50	4.28 ± 1.48	4.37 ± 1.41
Yoghurt				
A	9.0 ± 0.00	8.40 ± 0.41	8.20 ± 0.64	8.23 ± 0.55
B	8.9 ± 0.13	7.85 ± 0.41	7.30 ± 0.44	7.53 ± 0.56
C	7.7 ± 0.37	7.10 ± 0.43	6.75 ± 0.31	7.13 ± 0.32

plant made yoghurt further helps in minimizing syneresis.

Microbiological analysis

Total Viable Count: The total viable count of dahi and three brands of yoghurt i.e. A, B and C is shown in Table 2. The mean values of total viable count of dahi and yoghurt A, B and C was 7.34 x 10^7 cfu per ml, 5.61 x 10^7 cfu per ml, 3.31 x 10^7 cfu per ml and 6.34 x 10^7 cfu per ml with standard deviation of 1.57, 0.06, 0.09 and 0.04 respectively . These results are in line with the findings of Davis and Mclachlan (1974).

There was no significant variation in total viable count of different samples of plant made yoghurt as compared to dahi because defined starter culture is used (Kon, 1959) under proper conditions of fermentation for manufacture of yoghurt. But in case of dahi undefined wild starter culture is used in improper ratio and amount. It also contains heterogeneous mixture of lactic acid bacteria (Masud *et al.*, 1991) so total viable count as well as variation, was more in dahi samples.

Coliform Count: The coliform count of dahi and three brands of yoghurt i.e. A, B and C is shown in Table 2. The average coliform count of dahi and three brands of yoghurt A, B and C was 4.39 x 10^3 cfu per ml, 0 per ml, 0.71 x 10^3 cfu per ml and 3.39 x 10^3 cfu per ml with standard deviation of 1.08, 0, 0.96 and 0.5, respectively. In most of yoghurt samples coliform bacteria were absent due to pasteurization of pre-mix prior to its incubation and some samples of yoghurt contained less count of coliform. It might be probably due to contamination at storage and display/sale outlet. Similar results have been reported by Lopez *et al.* (1997) who reported low number of coliforms in yoghurt samples. Also brands in completely sealed containers had the best microbiological quality (Ibrahim *et al.*, 1989). But in case of dahi coliforms were present in all samples, which reflects highly poor hygienic conditions and improper sanitation during manufacturing of dahi.

Organoleptic evaluation: The organoleptic evaluation of dahi and three brands of yoghurt i.e. A, B and C is shown in Table 3. The mean values of over-all acceptability score of dahi and three brands of yoghurt were 4.37, 8.23, 7.53 and 7.13 with standard deviation of 1.41,0.55, 0.56 and 0.32, respectively. These results are different from those reported by Sarkar *et al.* (1996).

There was no significant variation observed in overall acceptability of A, B and C brands of yoghurt as compared to dahi.

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Milk Fat Production Trend and Effect of Season on it at Sree-Nagor Milk Shed Area under Milk Vita Throughout the Year

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Abstract: This study was undertaken to find out the milk fat production trend both in qualitative and quantitative aspect and seasonal effect on it at Sree-Nagor Milk Shed Area (MSA) under Milk Vita in Munshigonj, Bangladesh. The experiment was conducted during the period of 1995 to 1999. Season had the tremendous effect on milk yield (l/d). The milk production showed highest in the month of June, lowest in January, August, September and December; but in other months of the year it seemed almost similar. However, the average fat value of milk at Sree-Nagor MSA followed almost a specific trend throughout the year. Furthermore, the average fat value of Sree-nagor MSA was in-significant ($P>0.05$) between different months of the experimental period, but that of each year (1995-1999) between different months were highly significant ($P<0.01$), which means that season has large effect on fat value.

Key Words: Milk fat, milk shed area, milk vita

Introduction

Milk is the first food for the newly born human beings. To serve this purposes it is the food that contains all the nutrients, the newly born requires. Main components of milk are water (≈87%) and total solids (≈13%). The total solids is further divided into fat and solids- not- fat (SNF). The components of milk especially fat and SNF are acted as an indicator of the quality of milk. Milk fat production trend is a seasonal operation, depending on the availability of feed, climatological conditions and on tradition (Berg, 1988). The milk fat content appears to be more strongly influenced by "season" than other components (Johnson, 1978) relatively high fat per cent observed during the autumn of each year and during the summer months (Sargeant *et al*., 1998).

Fat contents exhibit a pronounced seasonal trend, being higher in winter than in summer season (Yadav *et al*., 1994). In the context of Sree-nagor MSA, it is very important to know the milk fat production trend. Because, milk producers get milk price from Milk Vita on the basis of milk fat content. So, the present research work was undertaken to investigate the following objectives: -

i) To know total milk fat production pattern throughout the year at Sree-nagor MSA.
ii) To study the effect of seasons/months on milk yield and milk fat production.

Materials and Methods

This study was conducted at Sree-Nagor milk shed area (MSA) during the period from January 1995 to December 1999.

Sree-Nagor MSA: Sree-nagor MSA is situated on the bank of Ichamoti River under Munshigonj district, Bangladesh. In October 1993, through Milk Vita`s own resources this plant was established primarily for chilling purposes of collected milk (8,000L/day). Presently, it is nourishing 20 active primary milk producing societies covering 3 Districts, 4 Upa-zillas , 14 Union , 25 Villages & 950 family members (Azad, 2001).

Animals: Cattle breeds are mostly Holstein Frisian. Some of them are Sahiwal and rests are so-called non-descriptive type (Local). As per cattle survey 2001, it possesses about 4,326 cattle head, which is producing milk of about 9,282 L/day.

Parameter studied: Total milk yields (L/d) and it components (g/kg) was observed from different societies of Sree-nagor MSA, and interviewing the randomly selected milk producers through consolidated questionnaire collected relevant information. From 20 primary milk-producing societies, 10 members were randomly selected for this purpose.

Fat value: Mixed milk samples were taken randomly from the different reception vat of the milk producers of different primary milk producing co-operative societies everyday after collection of milk at morning and at evening. Milk samples were collected at 1st to 8th, 9th to 16th, 17th to 24th, 25th to rest of the days of individual month and fat% of those samples were estimated in Milk Vita by commercial or Gerber method. Then, the estimated fat% was converted to g/kg as per research requirement. The values were calculated to obtain 4 mean values (Replication) for a month. These mean values were further averaged to obtain daily average value per month (Treatment). It had been continued from January 1995 to December 1999.

Table 1: Fat value (g/kg) of milk of Sree-nagor milk shed area during the period of 1995-1999

Month	Year					Mean
	1995	1996	1997	1998	1999	
January	40.6^{bc}	40.5^{cd}	48.0^{ab}	45.6^{a}	44.6^{a}	46.0
February	45.7^{ab}	45.5^{cd}	48.2^{ab}	44.2^{ab}	43.1^{b}	45.3
March	45.5^{ab}	44.6^{d}	48.1^{ab}	43.5^{bcd}	42.2^{bcde}	44.7
April	43.9^{cd}	46.0^{cd}	48.1^{ab}	43.2^{bcd}	41.0^{de}	44.4
May	43.9^{cd}	45.0^{cd}	47.3^{b}	47.7^{bc}	40.9^{e}	44.3
June	43.3^{d}	47.0^{bcd}	47.0^{b}	42.1^{cd}	42.0^{bcde}	44.2
July	43.7^{cd}	49.7^{a}	48.4^{ab}	41.9^{cd}	42.2^{bcde}	45.1
August	44.0^{cd}	49.0^{ab}	49.2^{a}	41.2^{d}	42.2^{bcde}	45.2
September	44.9^{bc}	47.3^{abc}	48.0^{ab}	42.3^{cd}	42.5^{bcd}	45.0
October	45.6^{ab}	47.7^{abc}	47.6^{ab}	43.0^{bcd}	41.5^{cde}	45.0
November	46.0^{ab}	47.5^{abc}	47.8^{ab}	42.3^{cd}	41.2^{cde}	44.9
December	46.4^{a}	47.4^{abc}	47.1^{b}	42.6^{bcd}	42.6^{bc}	45.2
Average	44.5	46.4	47.9	43.0	42.2	44.9
Level of significance	**	**	**	**	**	NS
LSD	1.093	0.222	1.170	1.617	1.280	-

Means with different superscript(s) in the same column differ significantly; *Significant at 5% level of significant **Significant at 1% level of significant; ^{NS} Non-significant; ^{LSD}Least significant differences between two means.

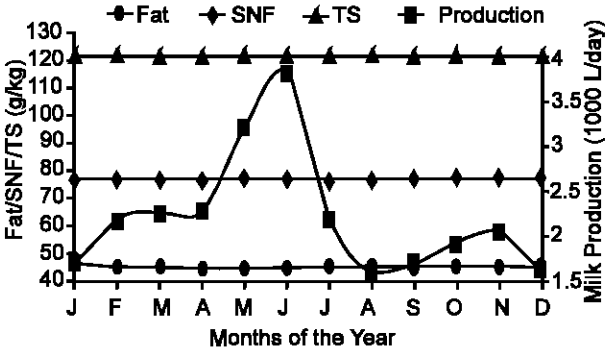

Fig. 1: Average milk production (L/D) with chemical qualities of Sree Nagor milk shed area during the period of 1995-99

Design of experiment: The collected data of the fat value were analyzed statistically by Completely Randomized Design (CRD) and significant differences among the treatment means were adjusted by Duncan`s New Multiple Range Test (Gomez and Gomez, 1984) using a MSTAT statistical package programme with the help of microcomputer.

Results and Discussion

The fat value (g/kg) of milk at Sree-Nagor milk shed area (SMA) during the period of 1995 to 1999 are presented in Table 1. Average fat value of Sree-nagor MSA did not differ significantly (P>0.05) between different months of the year (considering the whole experimental period), but the fat values of each year (1995 to 1999) between different months were differed significantly (P<0.01).

Considering the average fat values of 5 years, the highest (46.0 g/kg) fat value was observed in January (46.0 g/kg); lowest (44.2 g/kg) in June; and almost average (44.9 g/kg) in other months of the year. This variation might be happened due to the variation of green grass availability, seasonal effect, natural afford ability, disease infestation, and continuously using inadequate amount of roughages (straw) in the feeding system. Doll (1999) agreed with the present findings, who said that milk production became concentrated on competitive grassland locations.

Fig.1 represent the seasonal effect on chemical composition and production trend of milk at Sree Nagor, Munshigonj. The figure shows the message that season had little effect on average fat, solids-not-fat (SNF) and total solids (TS) value of milk (g/kg). The average yield (g/kg) of fat, SNF and TS were almost similar throughout the year. However, season had the tremendous effect on average milk yield (L/d). The milk production had been shown highest in the moth of June and lowest in January, August, September and December but in the other moths of the year it seemed almost similar. The average milk yield (L/d) was linearly increased from April, reached peak in June and then decreased. Per cent of fat production, some how, inversely related with the amount of milk production. The findings are in close agreement with that of Wood (1970), who showed that cyclic changes in day length, nutrition, or management may affect the milk production as well as milk fat production system in dairy cows. Yadov *et al.* (1994) also agreed with this result who stated that season have the large effect on milk yield as well as milk fat production.

Conclusion: From this study it was concluded that milk fat followed a specific trend and season have a tremendous effect on production system. Availability of forages may play the major role. So, aiming to gyre-up the economic status of the dairy co-operators of Sree-nagor MSA selective and planned technical know-how

and *ad-libitum* utilization of roughages should be introduced on priority basis in the traditional management of dairy enterprises.

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Status of Iodine Nutriture and Salt Iodization in Union Territory of Pondicherry, India

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Abstract: Deficiency of iodine causes a wide spectrum of disabilities including the implications on reproductive functions and lowering of IQ levels in school age children. The present study was conducted to assess the status of iodine nutriture and salt iodization in the entire Union Territory of Pondicherry, India. The study was conducted in all the four districts of Pondicherry. In each district 150 salt samples were collected by utilizing the uniform sampling methodology. The iodine content of salt samples was analyzed using the standard iodometric titration method. On the spot casual urine samples were collected from at least 80 children from the same school selected randomly The urinary iodine excretion (UIE) levels were analyzed using the wet digestion method It was observed that 59.7% of the families were consuming iodized salt with more than 5 ppm . It was found that 3 out of 4 districts had adequate iodine nutriture (median UIE levels more than 100 μg/l) possibly due to consumption of iodized salt by majority of the population. This study revealed the success of the salt iodization program in Pondicherry. However, there is a need for strengthening the existing monitoring and distribution system to ensure that adequately iodized salt is available for human consumption to eliminate IDD from the state.

Key Words : Iodine deficiency disorders, goitre, urinary iodine excretion levels, iodized salt

Introduction

India is the second most populated country in the world, with a population of more than 1000 million persons exposed to risk of Iodine Deficiency Disorders (IDD). Deficiency of iodine causes a wide spectrum of disabilities including the implications on reproductive functions and lowering of IQ levels in school age children (Vir, 1994).

Pondicherry is a coastal union territory (UT) and it is wrongly believed that populations residing in coastal areas do not suffer from IDD as they consume sea foods which are incorrectly believed to be rich in iodine. We conducted the present study to assess the status of iodine nutriture and salt iodization in the entire UT of Pondicherry.

Materials and Methods

The study was undertaken in all the 4 districts of Pondicherry during 2001. The guidelines recommended by WHO/UNICEF/ICCIDD for a rapid assessment of salt iodization in a district was adopted (WHO/UNICEF/ICCIDD, 1994). In each district 150 salt samples were collected by utilizing the uniform sampling methodology. All Senior Secondary schools in the district were enlisted and one school was randomly selected for the detailed study. All the children were briefed about the objectives of the study during the morning assembly. One hundred and fifty children belonging to different villages attending the school on the day of the survey were identified and were provided with auto seal polythene pouches with an identification slip. They were requested to bring four tea spoons of salt (about 20 g) from their family kitchen. The iodine content of salt samples was analyzed using the standard iodometric titration method (Karmarkar *et al.*, 1986).

At least 80 children from the same school were randomly selected and were requested to provide "on the spot" casual urine samples. Plastic bottles with screw caps were used to collect the urine samples. The samples were stored in the refrigerator until analysis. The urinary iodine excretion (UIE) levels were analyzed using the wet digestion method (Dunn *et al.*, 1993).

Results

A total of 600 salt samples were collected from four districts of Pondicherry. The district wise distribution of iodine content of salt is depicted in Table 1. It was observed that 59.7% of the families were consuming iodized salt with more than 5 ppm .

Table 2 depicts the district wise distribution of UIE levels. It was found that district Yanam had median UIE less than 100.0 μg/l along with more than 20% of the urine samples with less than 50 μg/l of iodine. These findings indicated deficient iodine nutriture in the district. In this district more than 75% of the families were found to be consuming salt with less than 5 ppm of iodine.

Table 1: Iodine content of salt samples collected at beneficiaries level in Pondicherry (n=600)

Name of the District	Iodine Content in ppm			
	N	<5	5-<15	15 & more
Karaikal	150	61 (40.7)	86 (57.3)	3 (2.0)
Pondicherry	150	100 (66.7)	40 (26.7)	10 (6.7)
Yanam	150	37 (24.7)	92 (61.3)	21(14.0)
Mahe	150	44 (29.3)	37 (24.7)	69 (46.0)
Total	600	242 (40.3)	255 (42.5)	103 (17.2)

Figures in parenthesis denote percentages

Table 2: Urinary Iodine Excretion levels in the study subjects in Pondicherry (n=377)

Name of the District	N	Median (μg/l)	UIE levels (μg/l)				Comments D/N
			<20.0	20.0- <50.0	50.0- <100.0	≥100.0	
Karaikal	80	150.0	0 (0.0)	3 (3.8)	9(11.3)	68(85.0)	N
Pondicherry	100	200.0	0 (0.0)	0 (0.0)	15(15.0)	85(85.0)	N
Yanam	100	65.0	5 (5.0)	27 (27.0)	34(34.0)	34(34.0)	D
Mahe	97	200.0	0 (0.0)	2 (2.1)	7(7.2)	88(90.7)	N
TOTAL	377		5 (1.2)	32 (8.5)	65(17.2)	275(73.0)	

Figures in parenthesis denote percentages, D= Deficient iodine nutriture, N= Normal iodine nutriture

Discussion

The results of the present study revealed that 60% of the population was consuming salt with more than 5 ppm of iodine. This finding showed that the population is purchasing and consuming iodized salt indicating the success of Universal Salt Iodization programme in Pondicherry. Earlier study conducted in Pondicherry, had reported low prevalence of goitre in school children as 2.6% (Kapil *et al.,* 1998a). Similar findings were reported from the coastal districts of Ernakulum where the prevalence of goiter was found to be 1.0% (Kapil *et al.*, 1998b). However, studies from other coastal districts of Kottayam, Portblair and Panaji had documented goitre prevalence rate of 7.05, 9.5 and 16.6 percent, respectively (Kapil *et al.*, 2002; Kapil *et al.,* 1996; Kapil *et al.*, 1998c) indicating endemicity of goiter. These studies have postulated that iodine deficiency was possibly due to goitrogens present in the food consumed by the coastal population in these districts as the percentage of population consuming iodized salt was 99.6, 51.1 and 99.5%, respectively.

WHO/UNICEF/ICCIDD have also recommended that no iodine deficiency is indicated in a population when median UIE level is 100.0 μg/l i.e. more than 50% of the urine samples have UIE level of 100.0 μg/l, and not more than 20% of samples have UIE level of 50.0 μg/l (WHO/UNICEF/ICCIDD, 1994). In the present study it was found that 3 out of 4 districts had adequate iodine nutriture (median UIE levels more than 100 μg/l) possibly due to consumption of iodized salt by majority of the population. Earlier study conducted in Pondicherry had also revealed a median UIE of 145.0 μg/l indicating no iodine deficiency (Kapil *et al.*, 1998a).

The findings of the present study revealed the success of the salt iodization program in Pondicherry as reflected by sufficient iodine nutriture in three out of four districts. However, there is a need for strengthening the existing monitoring system of the quality of salt to ensure that adequately iodized salt is available for human consumption in the union territory.

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Milk Production Trend of Milk Vita Throughout the Year

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Abstract: The present research work was undertaken to identify the quality milk production trend of Bangladesh Milk Production Co-operative Union Ltd. (Milk Vita) throughout the year. To achieve the objectives relevant data were collected during the period from January 1995 to December 1999 at different Milk Shed Area (MSA) of Milk Vita. Milk production trend of Baghabarighat and Takerhat MSA was differed significantly ($p<0.01$) among different months of each year during the experimental period and the average values of milk production of different years (1995-1999) were also differed significantly at 5 and 1% respectively. In Sree-Nagor, Manikgonj, Rangpur and Tangail MSA, milk production of each year differs significantly ($p<0.01$) and the average milk production values of different years did not differed significantly among different months of each year. From the mean values of five years record it was observed that milk production (TL/day) of Baghabarighat, Takerhat, Sree-Nagor, Manikgonj, Rangpur and Tangail MSA was 53.29, 4.19, 2.19, 2.47, 3.30 and 1.15 respectively. It was also revealed that the highest milk production was in February (10.01%) and lowest in September (6.46%) and milk production gradually increased from September to February which was indicated a specific milk trend throughout the year of Milk Vita. Fat and Solids-not-fat (SNF) production was little highest in December to April in Banghabarighat but same trend was not seen in all MSAs. From this study, it may be concluded that year round milk production of Milk Vita as well as Bangladesh were 9.97, 10.01, 9.20, 8.59, 8.17, 8.37, 7.27, 6.50, 6.46, 6.86, 8.73 and 9.88% respectively.

Key Words: Milk production, milk production trend, milk vita

Introduction

Milk is the first food for the newly born human beings. To serve its purpose it is a food that contains all the nutrients, the newly born requires. Milk production as well as chemical compositional trend is a seasonal operation, depending on the availability of feed, climatic conditions and on tradition (Berg, 1988). Bangladesh is deficit in milk and milk products. Domestic milk production (1.41MMT year^{-1}) represents only 12.77% of the actual need (11.04 MMTyear^{-1}). The gap between demand and production is 9.63 MMTyear^{-1}which is met by importing powder milk at the cost of valuable exchange. Bangladesh ranked 12th in the cattle population of the world and 3rd in the Asian countries arena, but it produces only a few portion of world's milk production. Where as India has become the world's largest milk producer, apart from the European Union (EU) and supplies gradually per capita more milk for their people i.e., 160ml day $^{-1}$, 164ml day^{-1}, and 166ml day^{-1} in 1988, 1989 and 1990 respectively (Sandstad, 1998). Quality milk production trend throughout the lactation period of a cow varies according to breed, feed, stage and number of lactation etc. and there is an inverse relationship observed between milk yield with its fat% mentioned by Banerjee (1995).

Different Milk Shed Areas (MSA) of Milk Vita has presently more than 300 primary milk producing societies. During the period of 1995 to 1999, milk production trend of Milk Vita has increased 12.36% by each year. The member of the society produces milk as a part of agricultural products like fish, paddy, poultry etc. But actual quality milk production trend of these MSAs as well as Milk Vita throughout the year is still unidentified which is very important for proper flurishment of dairying in Bangladesh indeed. For that reason, the present research was undertaken with the following objectives:

i To identify the milk production trend throughout the year of Milk Vita for initiating and organizing the prescribed developmental activities routinely.
ii To study the effect of different months/seasons throughout the year on quality milk production.

Materials and Methods

In this research work data on milk production, values of fat, solids-not-fat (SNF) and total solids (TS) were collected from January 1995 to December 1999 of different MSAs of Milk Vita namely: Baghabarighat, Takerhat, Sree-Nagor, Manikgonj, Rangpur and Tangail.

Milk Shed Area (MSA): Milk Vita has presently 10 MSAs that are placed in the different places of Bangladesh. Among 10 MSAs, 6 MSAs are encountered under this research work. Relevant information regarding type of animals, feeds and fodder situation, cattle population, nature of breeding practices, natural calamities etc. were collected from different MSAs through a consolidated short questionnaire.

Societies and animals: Milk Vita does have presently more than 300 primary milk producing societies. Average number of milking cows per member was 2.06 (Islam, 2000). The average number growth of membership per society was 39% in 1988-89, 52% in 1989-90 and 66% in 1990-91 session and the annual growth rate of membership per society was about 36% in 1989-90 and 26%in 1990-91(Ali *et al.*, 1996). Breeds of different MSAs were Sahiwal x Local, Sindhi x Local, Sahiwal x Sindhi and Local non-descriptive type and in some instances Holstein Friesian. Feeding and overall management practices of animals were mostly conventional and in some instance more or less innovative. Feeding pattern are mostly straw based and if concentrates are added as a supplementary item they use til oil cake, wheat bran, khesary bran, masturd oil cake etc.

Parameters studied: In the context of Bangladesh we have 4 seasons of the year viz: Monsoon (August-October), Winter (November-January), Spring (February-April) and Summer (May-June). Parameters relevant information was collected from the collection center of different MSAs and by interviewing the randomly selected milk producers from 5 primary societies from each of the MSA. So, the total number of societies and members was 30 and 120 respectively. Four parameters were studied in this experiment.

Milk production: Daily milk production was regularly recorded in a register book of quality control division of different MSAs of Milk Vita. Milk production from 1st to 8th, 9th to 16th, 17th to 24th and 25th to rest of the days of individual month were recorded. The values were calculated to obtain 4 mean values (Replication) for a month. These mean values were further averaged to obtain daily average per month (Treatment) for parameter.

Fat value: Whole milk was collected from the producers through co-operative venture. Mixed milk samples were taken randomly from the reception vat every day after milk collection at morning and evening for determining the chemical qualities of milk. Fat percentage of milk was estimated in Milk Vita by commercial (Gerber) method. Then, that was converted to g/kg. Daily average fat values (g/kg) per month were measured as mentioned in milk production.

Solids-not-fat (SNF) value: This was calculated according to the following formula (Mian, 1986).

$$SNF\ (\%) = CLR/4 + (1.2 \times Fat\%)$$

Raw data of SNF values were assembled by the same way as we did for fat value.

Total Solids (TS) Value: Total solids value of collected milk sample of different MSAs were collected from the sum of fat value and SNF value and these fat value were averaged by the same way as we did for other parameters.

Design of Experiment: Data obtained for different parameters were analyzed statistically by Completely Randomized Design (CRD) and significant differences among the treatment means were adjusted by Duncan`s New Multiple Range Test (Gomez and Gomez, 1984) using MSTAT statistical package program with the help of microcomputer.

Results and Discussions

Milk production: In Baghabarighat MSA highest and lowest milk production took place in December (68.02 TLday^{-1}) and August (41.14 TL day^{-1}) and average production was 53.29 TL day $^{-1}$ during the period from 1995 to 1999. Milk production of different years of this MSA differ significantly ($p<0.01$) among different months. Their (5 years) average value was also statistically significant ($p<0.05$) (Table 1).

In Baghabarighat MSA milk production gradually increases from October, stands pick point on December (Fig. 1) and begin to decrease from January to September. In the same way in respect of all MSAs milk production start to increase sharply from October to December. The milk production trend of Baghabarighat MSA and in respect of all MSAs clearly follows a specific trend. Razzak *et al.* (1995) found a unique relationship of milk production to the availability of feeds and fodder situation of Baghabarighat MSA, which supports of this research work. Variation in production for different months of the year and places might be due to availability of feeds and fodder, eco-environmental conditions and genetic make up of the individuals. Cady *et al.* (1983) also observed the effect of seasons or different months of the year on quality milk production and found that significant effects exist of season on milk production.

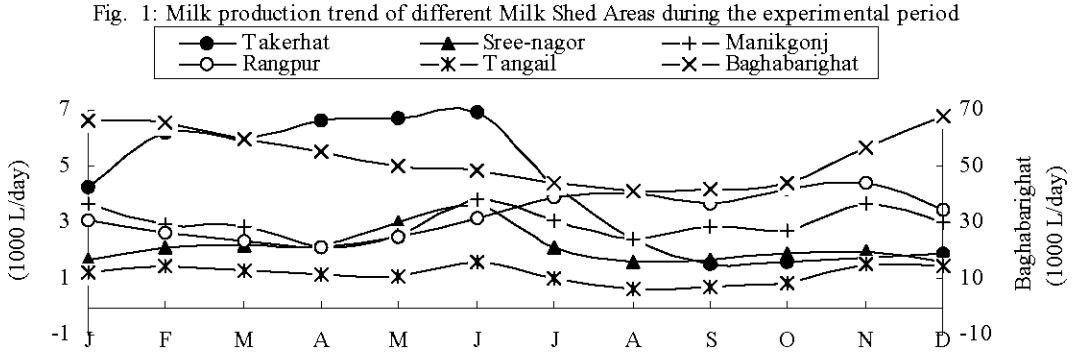
Fat value: Fat values of milk at Baghabarighat, Sree-Nagor, Manikgonj, Rangpur and Tangail MSA did not differ significantly but that of Takerhat MSA differs with others significantly ($p<0.05$) between different months of the year. Highest, lowest and average fat value was observed as 49.50g/kg (January), 47.28g/kg (October) and 48.50g/kg respectively. Two factors may involve for highest fat value in the month of January such as wide abundance of legume fodder and exercise or rearing facilities. But in October, cattle have to keep in confinement rearing due to seasonal flood, which may causes lowering fat value. On the other hand, in Milk Vita overall mean values of fat were found maximum, minimum and average as 45.43g/kg (September), 43.45 g/kg (April) and 44.52g/kg respectively (Table 2). The

Table 1: Average milk production day $^{-1}$ of different milk shed areas during the experimental period

Month	Mean (1000L day^{-1})						
	Baghabarighat	Takerhat	Sree-nagor	Manikgonj	Rangpur	Tangail	Mean/MSA
January	66.29 ^{ab}	4.29 ^{bcd}	1.65	3.67	3.06	1.21	13.36
February	65.23 ^{ab}	6.18 ^{ab}	2.11	2.96	2.64	1.44	13.42
March	59.44 ^{abc}	5.94 ^{ab}	2.17	2.83	2.34	1.27	12.33
April	54.96 ^{abc}	6.60 ^{ab}	2.19	2.11	2.09	1.14	11.52
May	49.82 ^{abc}	6.74 ^{a}	3.02	2.54	2.48	1.09	10.95
June	48.29 ^{bc}	6.91 ^{a}	3.59	3.80	3.18	1.56	11.22
July	44.07 ^{c}	4.34 ^{bc}	2.11	3.04	3.90	1.02	9.75
August	41.14 ^{c}	2.43 ^{cde}	1.58	2.43	4.05	0.60	8.71
September	41.55 ^{c}	1.54 ^{e}	1.67	2.83	3.67	0.72	8.66
October	43.93 ^{c}	1.60 ^{e}	1.87	2.74	4.18	0.82	9.19
November	56.78 ^{abc}	1.77 ^{e}	1.99	3.67	4.43	1.55	11.70
December	68.02 ^{a}	1.92 ^{de}	1.61	3.03	3.48	1.43	13.25
Average	53.29	4.19	2.13	2.97	3.30	1.15	11.17
Level of significance	*	**	NS	NS	NS	NS	NS
LSD	19.66	2.392					

Means with different superscript(s) within the same column differ significantly. *Significant at 5% level of significance. **Significant at 1% level of significance. ^{NS}Non-significant. ^{LSD}Least significant differences between two means.

Fig. 1: Milk production trend of different Milk Shed Areas during the experimental period



N.B : J,F.....D, indicates the name of different months of the year.

Table 2: Average Fat value day $^{-1}$ of different milk shed areas during the experimental period

Month	Mean (g/kg day^{-1})						
	Baghabarighat	Takerhat	Sree-nagor	Manikgonj	Rangpur	Tangail	Mean/MSA
January	49.5	37.20 ^{cd}	46.0	48.6	40.9	47.3	44.92
February	48.0	36.40 ^{cd}	45.3	48.4	40.5	44.6	43.87
March	48.9	35.40 ^{d}	44.7	48.3	39.7	44.6	43.60
April	48.6	36.20 ^{d}	44.4	48.1	39.2	44.2	43.45
May	49.3	37.70 ^{bcd}	44.3	48.1	39.3	46.0	44.12
June	48.9	39.00 ^{abcd}	44.2	47.8	39.8	44.4	44.02
July	48.7	41.00 ^{ab}	45.1	48.9	40.3	44.9	44.82
August	48.4	41.90 ^{a}	45.2	49.7	40.2	45.7	45.18
September	47.7	41.60 ^{a}	45.0	50.4	40.0	42.9	45.43
October	47.2	40.90 ^{ab}	45.0	50.2	40.5	46.9	45.12
November	47.5	39.50 ^{abc}	44.9	50.1	41.5	47.3	45.13
December	47.8	38.40 ^{abcd}	45.2	43.3	40.4	48.0	43.85
Average	48.4	38.80	44.9	48.9	40.1	46.0	44.52
Level of significance	NS	*	NS	NS	NS	NS	NS
LSD		0.3638					

Means with different superscript(s) in the same column differ significantly. *Significant at 5% level of significance. ^{NS} Non-significant ^{LSD}Least significant differences between two means.

Table 3: Average SNF values day^{-1} of different milk shed areas during the experimental period

Month	Mean (g/kg day^{-1})						
	Baghabarighat	Takerhat	Sree-nagor	Manikgonj	Rangpur	Tangail	Mean/MSA
January	79.0	71.01	76.0	75.5	75.0	74.0	75.09
February	79.2	71.67	76.0	74.6	74.9	73.2	74.93
March	79.0	71.67	76.3	75.1	74.9	73.3	75.05
April	78.6	71.89	76.5	74.8	74.1	73.3	74.87
May	78.6	71.84	77.1	74.7	74.4	73.4	75.01
June	78.7	71.43	76.6	74.5	74.4	73.2	74.81
July	78.8	71.17	75.7	74.1	74.4	73.4	74.60
August	78.5	71.28	75.7	74.8	73.7	74.5	74.75
September	77.6	71.27	76.3	75.0	73.7	74.5	74.73
October	77.7	71.30	76.6	74.6	74.0	74.5	74.78
November	78.7	71.03	76.7	74.3	74.5	73.6	74.81
December	77.3	71.29	76.6	74.3	74.4	74.2	74.68
Average	78.4	71.40	76.3	74.7	74.3	73.8	74.82
Level of significance	NS	NS	NS	NS	NS	NS	NS
LSD							

Means with different superscript(s) in the same column differ significantly. NS Non-significant. LSD Least significant differences between two means.

Table 4: Average TS values day^{-1} of different milk shed areas during the experimental period

Month	Mean (g/kg day^{-1})						
	Baghabarighat	Takerhat	Sree-nagor	Manikgonj	Rangpur	Tangail	Mean/MSA
January	127.6	107.86 ^{cd}	122.0	123.6	116.0	121.2	119.71
February	127.9	107.91 ^{cd}	121.5	123.2	115.4	117.8	118.95
March	127.8	107.22 ^{d}	120.9	125.7	114.5	117.9	119.00
April	126.9	108.25 ^{bcd}	121.2	123.0	113.2	117.5	118.34
May	129.5	109.86 ^{abcd}	121.3	122.8	113.6	119.4	119.41
June	128.5	111.04 ^{abcd}	121.3	122.3	114.3	118.3	119.29
July	127.7	112.51 ^{abc}	120.9	123.8	114.6	118.3	119.64
August	126.5	113.42 ^{a}	121.0	124.5	1136	120.2	119.87
September	126.0	112.97 ^{ab}	121.3	125.3	113.3	122.5	120.23
October	125.2	112.19 ^{abc}	121.7	124.7	114.8	121.4	120.00
November	125.8	110.29 ^{abcd}	121.8	124.1	115.6	120.9	119.75
December	126.5	109.66 ^{abc}	121.8	122.6	115.2	122.2	119.66
Average	127.0	110.27	121.4	123.8	114.5	114.8	119.46
Level of significance	NS	*	NS	NS	NS	NS	NS
LSD		4.854					

Means with different superscript(s) in the same column differ significantly. *Significant at 5% level of significance. NS Non-significant; LSD Least significant differences between two means.

average fat value during the period of 1995 to 1999 of Milk Vita was within the range of said by Judkins and Keener (1960). Fat production was highest in December to April in Baghabarighat MSA but not seen in other MSAs due to possibilities of adulteration of milk.

Solids-not-Fat (SNF) value: SNF value of Baghabarighat MSA differed significantly ($p<0.01$) among different months of different years, but their mean value did not differ. The difference between highest and lowest SNF value was 1.98g/kg. This figure is clearly denoting a specific trend of SNF production around the year. SNF production like fat was little highest in December to April in Baghabarighat but same trend was not seen in other MSAs. On the other hand, in respect of all MSAs highest, lowest and average SNF values were 75.09g/kg (January), 74.60g/kg (July) and 74.82g/kg respectively (Table 3). Variation of SNF value in respect of different months might be due to linearship of fat and SNF; types of feed and fodder availability, breeds etc. Jacobson (1936) and Jack *et al.* (1951) also found the same relationship as we observed in this research.

Total solids (TS) value: Total solids value of Baghabarighat MSA were differed more significantly ($p<0.01$) between different months but the average value

of different years were in-significant. In respect of all MSAs highest, lowest and average TS values were 120.23g/kg (September), 118.34g/kg (April) and 119.46g/kg respectively (Table 4). These values were not denoting a specific trend throughout the year. When milk production increases the TS values in general point of view decreases. Azad (1998) reported the TS values of Baghabarighat MSA were between 103.98g/kg to 151.40g/kg, which supports this research output.

Conclusions: From this research work it may be concluded that milk production, fat value and TS value of milk at Milk Vita follows a specific trend of production throughout the year. As per research findings, it was observed that highest milk production took place in February (10.01%) and lowest in September (6.46%) and milk production gradually increases from September to February. It clearly indicates that a specific quality milk production trend exist in Milk Vita around the year. Different MSA's of Milk Vita are placed in different ideal milk potential zones of Bangladesh. Therefore, Milk Vita's quality milk production trend throughout the year is actually presenting the picture of all over the country. The findings of this research may be authentically used for milk procurement budget preparation annually in Milk Vita as well as in Bangladesh in respect of consumer's demand. However, further study covering more potential areas on the same objectives is needed for a firm conclusion.

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The Benefit of Traditional Recipes - Boiled Legumes

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Introduction

For many years, chickpeas (*Vigna sinensis*) and various types of bean (*Phaseolus vulgaris*) have been staple foods for peoples around the Arabian Gulf. Generally, these legumes are washed to remove any field debris, and then soaked overnight in cold water. Next day, the peas and/or beans - often these dishes are a mixture of a number of types of legume - are boiled in salted water and, according to Musaiger (1993), sodium bicarbonate may be added to the boiling water to help soften them. The cooked legumes are then cooled, partly drained, and blended with olive oil, lemon juice and mixed spices before serving. The composition of the mixed spices - known locally as Ibzar - varies from family to family, but usually contains finely-ground black pepper, cumin, coriander and ginger as the important ingredients.

The final item is known in some regions as Foul Medammas and, while the moisture content is in the region of 70%, a typical portion (100 g) of the dish provides around 15 - 16 g carbohydrate, 6.0 g protein, 1.0 g fat and 0.75 g minerals, including 65 - 70 mg of calcium (Musaiger, 1993). As it is widely consumed across the Gulf Countries for breakfast or supper, it is an important dietary component, but also one that should present no microbial hazard to the consumer. Thus, boiling of the legumes eliminates any vegetative cells of pathogens that might be present and, as the dish is usually consumed shortly after cooking, spore-forming pathogens like *Bacillus cereus* will have no opportunity to grow and produce toxins.

However, times are changing. Younger members of a family may no longer join their parents for supper, but simply 'grab a bite' of something later in the evening. Such a 'snack' could well include a plate of Foul Medammas left over from an earlier meal. If the time between the cooking and consumption of the dish is short, or the Foul Medammas has been rapidly cooled and placed in a refrigerator, then the risk from spore-formers remains low but, if these conditions do not apply, then a traditionally safe food could become a hazard. Just as important as new eating patterns may be changes in the formulation of the dish, for Foul Medammas is now available in cans. This convenience no doubt has its advantages for students or single people living alone, and it is easy to imagine that such a person could warm the product in a microwave oven, eat a portion and leave the remainder for breakfast next morning. However, this proposed scenario leaves one question unanswered - is the microbiological security of the canned Foul Medammas the same as that of the traditional home-made product ?

In order to answer this question, the two types of Foul Medammas were 'challenged' with a mild pathogen that is widespread in kitchens, *Pseudomonas aeruginosa*. It is a typical Gram-negative, non-spore-forming, rod-shaped member of the *Pseudomonadaceae* and, if ingested in food or water at levels above 1.0 x 10^6 colony-forming units (cfu) g (or ml)$^{-1}$, it can colonise the intestine of even healthy individuals and cause diarrhoea (Rusin *et al.*, 1997); for immuno-compromised adults, the impact can be much more severe (Artenstein and Cross, 1993). As the pathogen can occur naturally in kitchen sinks and similar habitats, it could easily be 'splashed' into a bowl of food left standing nearby.

Consequently the aim of this project was to:

1. obtain samples of canned and fresh Foul Medammas
2. inoculate each type of product with *Ps. aeruginosa*
3. determine whether or not the pathogen would grow in either sample.

Table 1: Analyses of the canned and fresh samples of Foul Medammas (see text for details); figures for total solids as g 100^{-1}

Sample	pH	Total Solids	Water Activity
Fresh	5.0	32.1	0.99
Canned	5.9	21.0	1.0

Materials and Methods

The culture of *Ps. aeruginosa* was grown on slopes of Brain Heart Infusion Agar (Oxoid Code No. CM375, Unipath Ltd., Basingstoke, Hampshire, UK), and loopfuls were added to a tube of sterile saline solution until the optical density indicated a cell count of 1.5 x 10^8 cfu ml^{-1} (Anon., 2001). This suspension was further diluted to give a solution containing 1.5 x 10^6 cfu ml^{-1}.

The canned samples of Foul Medammas were purchased from a local shop, while the fresh product came from a restaurant specializing in Arabic food; the fresh samples were used with 24 h of preparation. Sub-samples (10 g) of the two types of Foul Medammas were taken for analysis for total solids, pH (Kirk and Sawyer, 1991) and water activity (Novatron Meter, Horsham, Sussex, UK). Two bulk samples (200 g) of each type of product were tranferred to stomacher bags, and 1 ml of the dilute culture was added to each bag to give an initial cell count of approximately 7,500 cfu g^{-1}.

Table 2: Total colony counts of *Ps. aeruginosa* in the canned and fresh samples of Foul Medammas stored at 4 or 25 °C for the times shown; all figures as mean cfu g^{-1} of product as consumed, and the trial was repeated on two occasions

Time (hours)	Temperature			
	4 °C		25 °C	
	Fresh	Canned	Fresh	Canned
0	7.0×10^3	2.0×10^3	7.0×10^3	2.0×10^3
4	6.2×10^3	2.2×10^3	6.0×10^3	3.7×10^3
8	6.0×10^3	2.2×10^3	6.0×10^3	1.6×10^5
12	6.0×10^3	2.3×10^3	6.0×10^3	1.7×10^6

After blending in the Stomacher (Unilever, Sharnbrook, Bedfordshire, UK), one bag of each type was placed at room temperature (~ 25 °C) and one at 4 °C. Each bag was sampled at 4, 8 and 12 hours by removing a sub-sample (1 g), creating a dilution series in quarter-strength Ringer's Solution (Oxoid Code No. BR52) down to 10^{-7}, and spreading 0.1 ml samples onto pre-poured plates of *Pseudomonas* Agar selective for *Ps. aeruginosa* (Oxoid Code No. CM559/SR102). Counts were taken after 48 h and recorded as cfu g^{-1} of product; the entire trial was repeated twice.

Results and Discussion

The basic analyses shown in Table 1 confirm that the restaurant-made product has a solids content close to that proposed by Musaiger (1993), but the canned Foul Medammas had a solids content more than 10% lower. The high moisture contents were reflected also in the water activities, and the canned product gave a quite exceptional value for a food (pure water has an A_w of 1.0). Bearing in mind that values of available water above 0.93 in a food are conducive to bacterial activity (Corry, 1979), it is not surprising that Foul Medammas could be susceptible to bacterial spoilage. Acidity is another factor that can control microbial growth, and it is notable that the canned product has a pH of 5.9 compared with 5.0 for the fresh Foul Medammas. As *Ps. aeruginosa*, as well as other Gram-negative pathogens like *Salmonella* spp., usually grow best at pH 6 - 7 and are inhibited around pH 4.5, it is evident that the fresh product will be less likely to support extensive microbial growth.

This view was borne out by the results shown in Table 2, and contrast between the growth of *Ps. aeruginosa* in the fresh and canned products was quite remarkable. At 4 °C, the low temperature controlled the growth of the pathogen in both products but, when this restraint was removed at 25 °C, the organism grew extremely rapidly in the canned product. Indeed, after 12 h, the cell count of *Ps. aeruginosa* had exceeded the minimum infective dose, even though neither the appearance nor smell of the material revealed the extent of the microbial activity that had occurred. By contrast, the fresh product did not support the growth of the pathogen at all at 25 °C.

A further comparison of the two types of product revealed some possible reason(s) for the differences in the behaviour of *Ps. aeruginosa*, for while both materials contained salt (~2.0 g $100g^{-1}$), only the fresh Foul Medammas contained olive oil (~ 3 g 100 g^{-1}) and lemon juice (~ 8 g 100 g^{-1}). The presence of lemon juice explains the lower and more inhibitory pH of the traditional product, but equally important may the antimicrobial activity of the olive oil (Raina, 1993; Keceli and Robinson, 2002). Thus, a number of the phenolic compounds found in virgin olive oil are inhibitory to species of bacteria and yeasts, especially at low pH, and it may be that the antimicrobial effects of reduced pH, salt and phenolic agents combined to prevent the growth of *Ps. aeruginosa*. In the canned product, the absence of the 'traditional' ingredients allowed the organism to grow without restraint.

Obviously it can be argued that dishes like Foul Medammas should not be left at ambient temperature anyway, but what is really relevant is the contrast between the traditional and 'modern' products with respect to their ability to support undesirable microbial growth. Thus, many traditional foods have 'evolved' rather than been formulated by a chef working in a modern kitchen and, as a result, ingredients have been incorporated to give both pleasant characteristics to the dish and, albeit by chance perhaps, an element of 'safety' for the consumer. This inherent stability of traditional recipes is not uncommon (Campbell-Platt, 1987) and to forget this lesson could well put consumers at risk.

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Natamycin In Ripening Cheeses

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Abstract: "Delvocid" (Gist Brocades nv.) a preparation containing the natamycin, a substance used in the therapeutics, is used in cheese making to protect a cheese surface against mould development. This preparation is added either to polyvinyl acetate (PVA) used for cheese coating or as an aqueous suspension in which cheese is immersed before the ripening. The natamycin content in the outer layer of cheese was determinated by a spectrophotometric method. During maturation of cheese coated with natamycin-containing polyvinyl acetate, the natamycin did not migrate into the cheese. The natamycine concentration in cheeses that had been immersed in an aqueous solution of natamycin was considerably higher than that in PVA-coated cheeses and was related to the type of cheese, concentration of the "Delvocid" preparation, the length of time for which the cheese was held in solution.

Key Words: Cheese, natamycin, migration into the cheese, mould development

Introduction

Environmental conditions prevailing during cheese ripening, combined with the composition of the cheese often create possibilities for extensive development of mould on cheeses surfaces, which reduces considerably its quality. As a result of mould growth, aflatoxins may be produced in cheese, rendering it unfit for human consumption (Kiermeier and Zeier, 1975; F.I.L., 1977). Therefore, during ripening, cheeses are protected against development of mould by coating of their surfaces a protective layer.

The use of "Delvocid" (Gist Brocades NV), containing 50% natamycin has considerably facilitated protection of cheeses against mould growth during ripening. "Delvocid" may either be added to brine, used as an aqueous suspension for dipping of cheeses or added to polyvinyl acetate used to coat the cheese.

Natamycin, at a concentration considerably lower than other known fungicides, destroys both mould hyphae and spores (Moll, 1966; Shahani *et al*., 1985). Until now, cheeses have been protected against mould growth by treatment with propionic acid (Kujawski *et al*., 1983), sodium, calcium or potassium sorbate (Moll, 1966; Lück, 1972; 1975).

The inhibitory concentration of sorbate is as high as 300 mg/dm² of cheese surface while the permissible concentration of natamycin is only 2 mg/dm² (WHO, 1976). Sorbate diffuses into cheese and affects its organoleptic properties (De Ruig and Von Der Berg, 1985) whereas the migration of natamycin is negligent, and when used in polyvinyl acetate, it remains on a surface of the cheese (Kiermeier and Zeier, 1975; Reps *et al*., 1987).

Because natamycin is used as a human medicine, it is necessary to determine its concentration in various types of cheese and the rate of its decay.

In the present study, the suitability of "Delvocid" for inhibition of mould on cheese surfaces was investigated. The experiments were made on Salami (Tilsit type cheese) and Jeziorański (Münster type cheese) cheeses ripened on dry surface for four weeks.

Material and Methods

To determine the suitability of "Delvocid" as a fungicide for cheese, cheeses were subjected to one of the following treatments:

* Cheeses Salami and Jeziorański were dipped into 0.2 and 0.4% aqueous suspension of the preparation for 60 sec, before brining.
* Salami and Jeziorański cheeses were salted in brine containing 0.2% "Delvocid".
* Salami and Jeziorański cheeses were immersed for 30 and 60 sec in a 0.2 and 0.4% aqueous suspension of "Delvocid" after brining.
* In order to examine an influence of cheese rind on the natamycin content in the outer layers of the cheeses, standard Salami cheese and Salami with the rind removed, were immersed for 60 sec in a 0.4% aqueous suspension of "Delvocid".
* Gouda type cheeses were immersed for 30 sec in a 0.2% aqueous suspension of "Delvocid" and packed in Cryovac bags while wet or after drying the surface. Cheese was also packed in bags which had been dipped in a 0.2% aqueous suspension of "Delvocid".
* Edam cheeses, after brining, were coated with one, two and three layers of polyvinyl acetate containing 0.1% of "Delvocid". After one week of ripening, half of the cheeses from each lot were covered with cheese wax.

During the ripening of cheeses, the effectiveness of antifungal action of natamycin was observed (compared

Table 1: Natamycin concentration in 1 mm thick surface layer of cheeses, immersed in an aqueous suspension of "Delvocid" before brining

Concentration of "Delvocid" %	Duration of ripening (days)	Salami cheese	Jezioranski cheese
		Natamycin concentration (mg/dm^2)	
0.2	4	0.18	0.22
	9	0.16	0.15
	14	0.10	0.00
	19	0.00	0.00
	24	0.00	0.00
	28	0.00	0.00
0.4	4	0.36	0.37
	9	0.31	0.31
	14	0.18	0.31
	19	0.11	0.14
	24	0.05	0.00
	28	0.00	0.00

Table 2: Influence of rind on the natamycin concentration in the surface layer of Salami cheeses immersed for 60 Sec in an aqueous suspension of "Delvocid"

State of cheese surface	Duration of ripening (days)	Layer of cheese				
		0-1	1-2	2-3	3-4	4-5
		Natamycin concentration (mg/dm^2)				
Control cheese	4	1.66	0.14	0.0	0.0	0.0
	14	1.39	0.27	0.0	0.0	0.0
Cheese after rind removal	4	4.11	1.85	1.11	0.48	0.0
	14	3.23	2.01	1.82	0.94	0.45

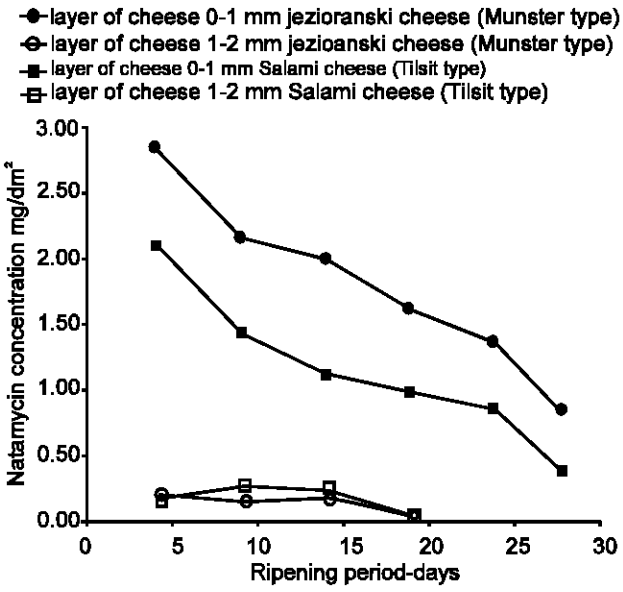

Fig. 1: Natamycin concentration in the surface layer of cheese after salting in brine containing 0.2 % "Delvocid"

to the control cheeses) and its concentration in five consecutive, 1 mm-thick layers of cheese was determined, by the spectrophotometric method (De Ruig and Von Der Berg, 1985).

Results and Discussion

In cheeses, that had been immersed in and aqueous suspension of "Delvocid" before brining the concentration of natamycin on the surface of cheeses was insignificant (Table 1), presumably due to washing out the natamycin (which is insoluble in water) from the surface of cheeses by the brine. In spite of the low concentration of natamycin, little mould development was observed after two weeks of ripening whereas mould development was observed considerably earlier on control cheeses.

It should be mentioned that under the industrial conditions, the concentration of natamycin capable of inhibiting mould development on the surface of cheese may depend on the type of cheese and environmental conditions in the ripening room.

The concentration of natamycin in the outermost 1 mm-thick outer layer of Jeziorański and Salami cheeses after salting in natamycin-containing brine was 2.84 and 2.09

Table 3: Natamycin concentration in surface layers of Gouda cheese, ripening in Cryovac plastic bags

Procedure	Duration of ripening (days)	Layer (mm)	Natamycin concentration (mg/dm^2)
Cheese packed in film in a wet state	21	0-1	0.90
		0-2	0.00
	26	0-1	0.64
		1-2	0.00
	32	0-1	0.31
		1-2	0.00
	38	0-1	0.11
		1-2	0.00
	42	0-1	0.00
		1-2	0.00
Cheese packed in film after drying of its surface	21	0-1	0.32
		1-2	0.00
	26	0-1	0.12
		1-2	0.00
	32	0-1	0.11
		1-2	0.00
	38	0-1	0.00
		1-2	0.00
	42	0-1	0.00
		1-2	0.00
Cheese packed in film soaked in an 0.2 % aqueous suspension of "Delvocid"	21	0-1	0.15
		1-2	0.00
	26	0-1	0.08
		1-2	0.00
	32	0-1	0.00
		1-2	0.00
	38	0-1	0.00
		1-2	0.00
	42	0-1	0.00
		1-2	0.00

Table 4: Changes in natamycin concentration in surface layer of Edam cheese

Duration of ripening in PVA film (days)	Cheese coated with one layer of PVA		Cheese coated with two layers of plastic		Cheese coated with three layers of plastic	
	PVA	PVA+wax	PVA	PVA+wax	PVA	PVA+wax
	Natamycin concentration (mg/dm^2)					
4	0.43	0.43	0.68	0.68	0.98	0.98
8	0.27	0.27	0.38	0.28	0.63	0.63
11	0.18	0.23	0.26	0.37	0.54	0.48
15	0.14	0.20	0.17	0.28	0.38	0.41
18	0.00	0.16	0.00	0.21	0.25	0.26
21		0.00		0.16	0.14	0.17
23				0.00	0.00	0.11
25						0.00

mg/dm² respectively (Fig. 1), which exceeded the permissible concentration. During ripening, a rapid decline in the concentration of natamycin in cheese was observed. On the 28th day of ripening, before the cheeses were distributed, the concentration of natamycin in Salami cheese was 0.32 mg/dm², and in Jeziorański cheese was 0.79 mg/dm². Traces of natamycin were also found in the second 1 mm layer of cheeses from the surface (1-2 mm) (Fig. 1). Throughout ripening, moulds did not grow on the surface of the experimental cheeses.

In Jeziorański cheese, that was immersed in an

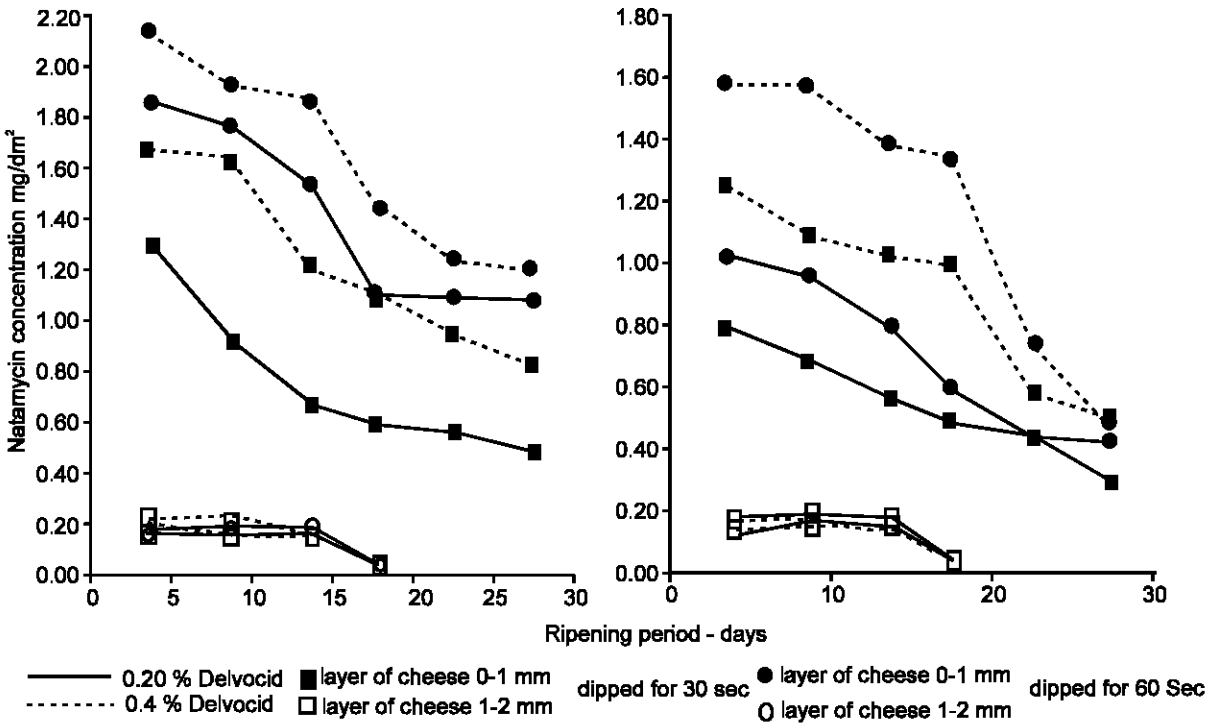

Fig. 2: Natamycin content in surface layer of cheeses, after salting, dipped in aqueous suspension of the "Delvocid"

aqueous suspension of "Delvocid" after brining, the natamycin content was higher than that in Salami cheeses that had been treated similarly (Fig. 2). However, only in case of cheese that had been immersed for 60 sec in a 0.4% suspension of "Delvocid", did the natamycin level exceeded the permissible concentration during the initial period of ripening. During ripening, inactivation of natamycin was observed and was more intensive in Salami cheese. In the second layer from the outside of the cheese, the natamycin content was insignificant and by the 18th day of ripening had decreased to non-detectable levels. The higher concentration of natamycin on the surface of Jeziorański cheese can be explained by the fact that its rind is more porous (non-pressed cheese) than that in Salami cheese (pressed cheese).

During the ripening of Jeziorański and Salami cheeses, the development of mould on their surface was not observed. Other authors (Gripon and Bergere, 1972; Engel *et al.*, 1983) also showed that the concentration of natamycin in the outer layer of cheese and the depth of its migration depended on the type of cheese and on the duration of immersion in the suspension of "Delvocid".

A major influence of the rind on the concentration of natamycin in cheeses, as well as on the depths of its migration, was confirmed (Table 2). On the 4th day of ripening in the cheeses from which the rind had been removed, the concentration of natamycin in the outermost layer of cheeses was by 2.5 times higher than that in cheeses with the rind. In rindless cheeses, the migration of natamycin to a depth of 5 mm was observed.

The result of the study showed that natamycin protects the surface of cheese against the development of mould. For the efficient protection of the surface of Salami and Jeziorański cheeses it is recommended to salt them in brine containing 0.2% "Delvocid" or to immerse them for 30 sec in a 0.4% aqueous suspension of "Delvocid". The suspension of "Delvocid" in water or brine may be used for a long period.

The concentration of natamycin in the outer layers of cheese depends on the type of cheese, rind performance and the method of application of "Delvocid". During ripening, natamycin adsorbed in the outer layer of the cheeses is inactivated quickly. After four weeks of ripening, before the distribution of cheeses to retailers, natamycin was present only, in the negligent quantities, in 1-mm thick outer layer of the cheeses.

Immersing Gouda cheese for 30 sec in a 0.2% aqueous suspension of "Delvocid" before packing in Cryovac film, or soaking only of the bags in the above mentioned suspension effectively protected the surface of the cheese from the development of mould, even when the coating was damaged.

Natamycin present in "Delvocid" did not migrate into the Gouda cheese (Table 3). After the ripening period, requested by Polish quality standards, natamycin was not found in cheeses.

In order to protect the surface of cheeses from the mould growth, polyvinyl acetate (PVA) containing 0.05 % natamycin is usually applied. In Edam cheeses, coated with one, two, or three layers of PVA, the concentration of natamycin in the 1-mm outer layer of cheese was 0.43, 0.68 and 0.98 mg/dm² of cheese, respectively (Table 4). Natamycin was not present in the second layer of cheese.

In the cheeses coated with one or two layers of PVA, natamycin was present for 15-18 days of ripening and when three layers of PVA were applied, it was present for 21-23 days. Early additional coating of cheeses with wax prolongated by a few days the presence of natamycin in cheese.

After from five to six weeks of ripening period cheeses did not contain natamycin.

Polyvinyl acetate containing of 0.05% natamycin effectively protected the surface of cheeses against the development of undesirable moulds and simplified handling of cheese during the ripening period.

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