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CONTENT

SHORT COMMUNICATION

BREAD CONTAMINATION WITH FUNGUS

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ABSTRACT

Mouldiness is one of the most common microbiological defects, found in bakery industry. It is estimated that approximately 1-5% of the bread production goes wrong due to fungi activity. Referring to bread, mold contamination determines not only changes in color, taste, but also loss of the food quality as a result of possible formation of mycotoxins. Mouldiness is caused by external contamination of bread after baking, because the existing spores in flour during a normal technological process don't have any multiplication conditions, and during baking they are destroyed. Bread contamination with molds, may occur in the following steps: the transportation of bread; during cooling and storage; while cutting and packing (optional operation).

Propagation of mold spores and contamination of bread can take place indirectly by air in the storage room (the air) or by direct contact with boxes, crates, hauling equipment etc. Among the molds that frequently contaminate bread during storage, there are the following common species and genera: *Alternaria, Aspergillus, Cladosporium, Geotrichum candidum, Mucor, Neuropspora sitophila, Penicillium, Rhizopus stolonifera, Thamnidium elegans*. By the development of other fungi superficial pigmentation are produced, which only affects the commercial appearance of bread. Under these/the given circumstances, the development of *Geotrichum auranticum* and *Thamnidium auranticum*, gives a red-gold coloring of the bread in the colonies' development surface.[1]

Keywords*: mycotoxins, spores, changes, Penicillium.*

1. Introduction

Bread mouldiness occurs when the bread is stored under high relative humidity and high relative temperature. The colour of molds that grow on bread varies from white, golden yellow to green-gray, depending on the species and the degree of sporulation.[2]

The bread with moldy smell and taste is not to be used.

This determination intended to identify the species of fungi and the influence of temperature and type of flour on the bread. The study was conducted on two types of bread: enriched in fibers and bread from white flour. For the bread contamination we used two species of fungi, both belonging to the genus Penicillium.

Moulds were isolated from moldy orange and bread.

2. Materials and methods

Molds were isolated from the moldy orange and bread. We prepared spores suspensions in water (one more concentrated, another thinner). Final suspensions of spores contained ~ $47 \bullet 10^3$ cfu/ml as measured by the cytometer. They were then poured into glass tubes and stored at refrigeration temperature. We drew two diagonals on the surface of the two slices of bread. We dosed 5 ml of each solution with an automatic pipette and we inoculated the sample into bread.

The bread was introduced into the incubator for seven days, one being maintained at 25 ° C and the other at 19 °C.

After 7 days, we counted the mold spores grown (developed) into the two types of bread with the cytometer, Tables 1-4.

Counting microorganisms by direct microscopic examination using the cytometer. The mold spores can be counted by direct microscopic examination, using a cytometer. A cytometer is a thick glass slide, with three separate platforms interconnected by channels in the glass. The central platform is subsided of the other two with a height of 0.1 - 0.2 mm (included in each room).The central platform is engraved with a network of perpendicular lines, which delimitates a given area divided by perpendicular lines in the square-shaped microcells (elementary squares).

The differentiation between types of cytometers consists in the variable size (amount) of a basicsquare.[4]

Cell number is calculated by:

$$\frac{Total\ number\ of\ cells\ counted\ in\ 9\ squares}{Total\ number\ of\ squares\ counted(9\ squares)} \times Dilution \times (1 \times 10^4)$$

3. Results and discussions

By virtue of the following charts, we observed, Figures1, 2, 5:

-the bread enriched in fibers is less contaminated than the one obtained from white flour;

-the concentrated suspension used in bread contamination, causes a higher degree of spores multiplication, so we obtain a deeper contamination than in the diluted one;

- at 25° C, the number of spores is major (bigger) than at 19°C, so the bread is more contaminated.

Table 1. *Bread enriched with fibers incubate at 25°C*

The provenance (origin)of mold spores	Orange (concentrated suspension)	Orange (diluted suspension)	Bread (concentrated suspension)	Bread (diluted suspension)
Spores number ($\cdot 10^3$)	11	7,66	11,77	9,77

Table 2 .*Bread enriched with fibers incubate at 19°C*

The provenance (origin) of mold spores	Orange (concentrated suspension)	Orange (diluted suspension)	Bread (concentrated suspension)	Bread (diluted suspension)
Spores number ($\cdot 10^3$)	8,55	6,66	9,33	8,66

Table 3. *Bread from white flour incubate at 25°C*

The provenance (origin) of mold spores	Orange (concentrated suspension)	Orange (diluted suspension)	Bread (concentrated suspension)	Bread (diluted suspension)
Spores number ($\cdot 10^3$)	41	15	43,11	27,33

Table 4. *Bread from white flour incubate at 19°C*

The provenance (origin) of mold spores	Orange (concentrated suspension)	Orange (diluted suspension)	Bread (concentrated suspension)	Bread (diluted suspension)
Spores number ($\cdot 10^3$)	28,33	10,88	33,88	19

As the temperature increases we observe that mold spores coming from the moldy bread multiply in a larger number than mold spores coming from the moldy orange.

In the wake of microscopic examination of mold spores coming from the moldy bread *Penicillium* genus has been identified, species brevicompactum.

Penicilllium brevicompactum proved to be toxic when it is accumulated in cereals. It inhibits the growth of peas, vetch and wheat in vitro. It may cause deterioration and subsequent growth inhibition on stored ***Gladiolus bulbs***. It can grow anaerobically in a nitrogen atmosphere, but under these circumstances it requires biotin and thiamine. Positive evidence has been obtained for the ability to decompose starch, tannin, protein, cellulose, pectin and xylan. This mold can use hydrocarbons from fuel oil and it is also reported to grow on various plastic polymers. Organic acids are secreted in such amount of quantities that they can lead to metal corrosion.

In the wake of microscopic examination of mold spores coming from the rotten(moldy) orange the genus *Penicillium* was identified, species digitatum and italicum.

Penicillium digitatum is responsible for more than 80% of the citrus mouldiness; all species and varieties are susceptible. This mold exists in the entire world. It is present at any time during the whole process from the orchard to the refrigerator, through packing stations, vehicles, warehouses and shops. *Penicillium digitatum* is responsible for "green speck (mold)" of citrus, named this way because of the colour of its spores, which in late stages covers the entire surface of the affected fruit. It is strictly a surface parasite, unable to penetrate intact epidermis (outer skin). There are contamination opportunities in the orchard, but most infections occur in harvesting, packaging and marketing and later stages of consumption.

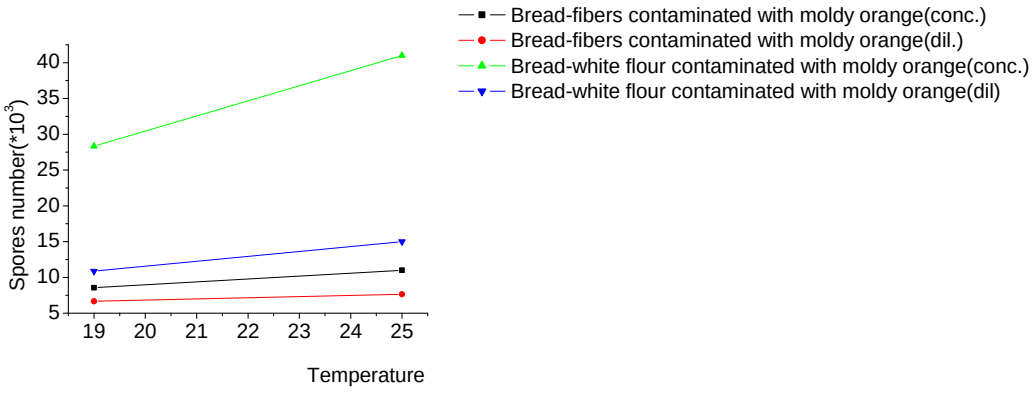

Figure1. The variation (fluctuation) of spores number which contaminate the bread from moldy orange

Initially, the affected area becomes soft, and then it develops a thin bright white coloured mycelium, to increase regularly, circularly, whose center will be covered with green spores.

In the advanced stages, green spores cover the entire surface of the fruit, epidermis (outer skin), flavedo and albedo, and the pulp is completely contaminated.

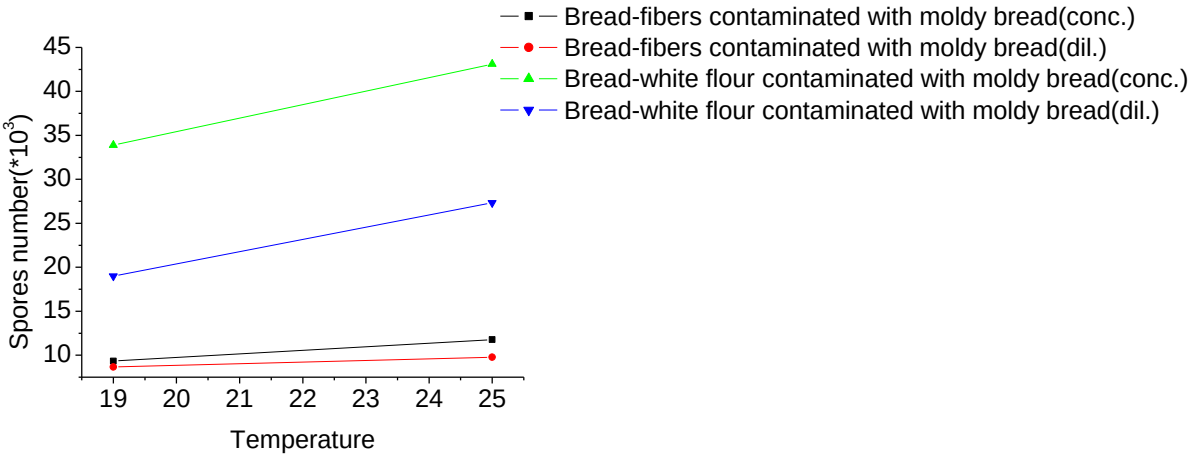

Figure 2. The variation (fluctuation) of spores number which contaminate the bread from moldy bread

Since the beginning of contamination, the fruit has an undesirable flavour.

The spores are easily carried by air circulation and colonize the already damaged fruits. *Penicillium digitatum* follows a well-defined sequence, it must degrade (break) the flavedo before the albedo and the pulp, the proof is that it can attack a superficially peeled fruit (flavedo), keeping only the albedo. The way *Penicillium digitatum* works is by producing ethylene, which accelerate fruit ripening.

Penicillium digitatum remains active at normal storage temperatures.

Penicillium italicum is responsible for "blue speck (mold)" of citrus, called this way because of the color of its spores. Its spores are present throughout the process flow, from packaging to consumption. The peel becomes soft, then a white fluff which covers quickly with blue mold spores appears. The flesh is simultaneously infected and the fruit, even partially contaminated, becomes inedible. The spores are easily transported from one fruit to another, and the mold spreads, also in direct contact. The two species may coexist on the same fruit, *Penicillium italicum* sometimes overlaps *Penicillim digitatum*, but not vice versa.[4]

4. Conclusions

Fungus develops in a high humidity environment, about 80% and at the temperature of 19-25 °C. Explanation: The peel is wet enough for spores to germinate, to multiply and form hyphae that penetrates the bread-crumb. The process is favoured by the waterproof packing bags.

Moldy bread loses weight and change the appearance both inside and outside, because of the mold colonies which have different colors: white, greenish, blackish, yellowish. As micotoxigene mold colonies grow, the

amount of mycotoxins in food increases. Moldy bread must be excluded from both human and animal nutrition.

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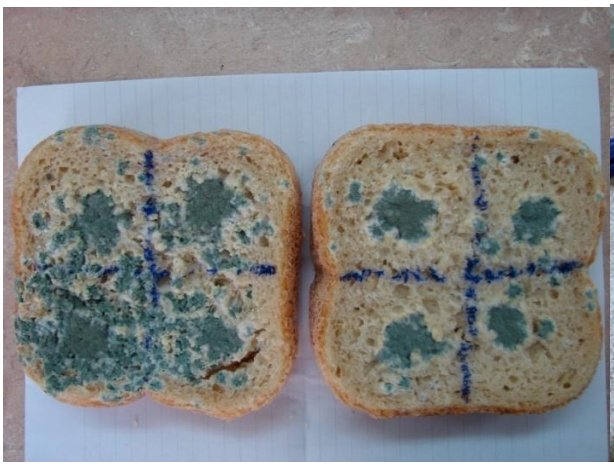

Figure 3. White bread contaminated with fungus fungus

Figure 4. Bread enriched with fibers contaminated with

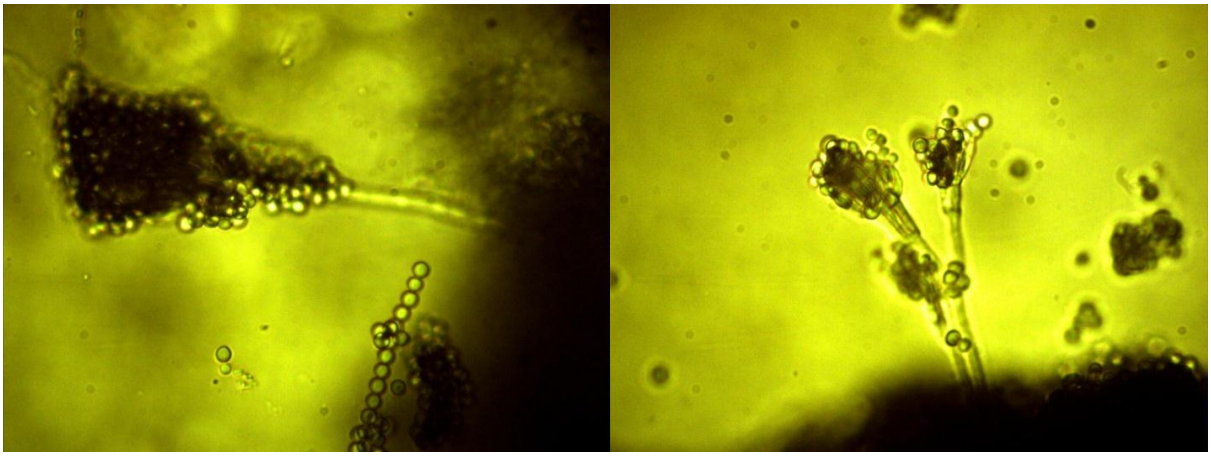

Figure 5. Penicillium brevicompactum

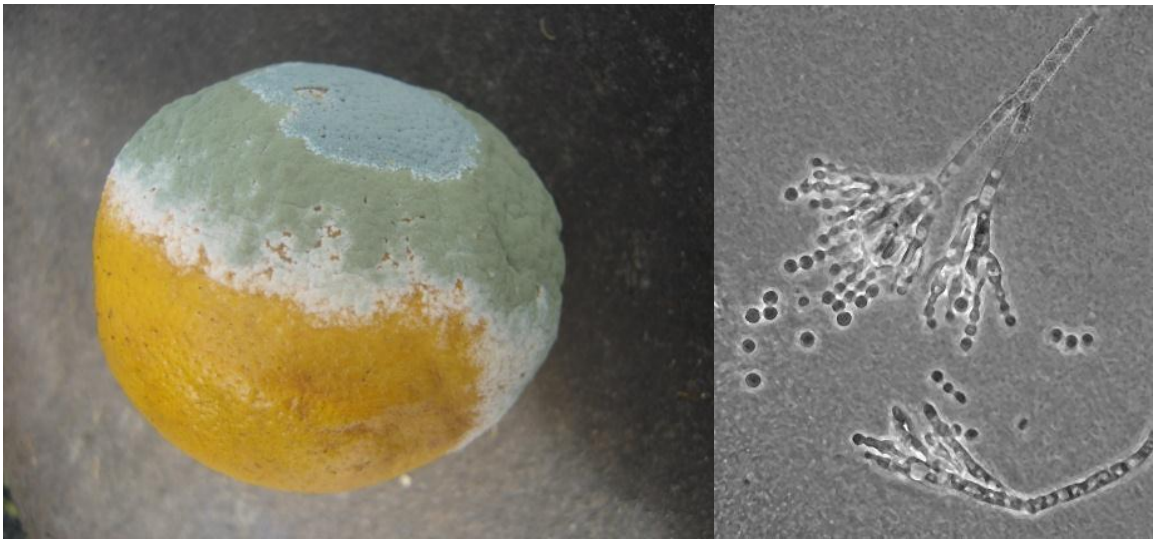

Figure 6. Orange contaminated with P. digitatum and P. italicum

Figure 7. Penicillium digitatum

PHYSICOCHEMICAL AND SENSORY CHARACTERISTICS OF CALCIUM-ENRICHED SOY-RED RICE MILK

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ABSTRACT

A study on physicochemical and sensory characteristics of Ca-enriched soy-red rice milk has been done. Stabilized-Calcium Carbonate at different level of 0.1, 0.2, 0.3, 0.4 and 0.5% w/v was added into soy-red rice milk. Viscosity of Ca-enriched soy-red rice milks were in a range of 125 and 300 cP. The viscosity was higher as higher level of stabilized-Calcium Carbonate added. Ca-enriched soy-red rice milk at all stabilized-Calcium Carbonate level has an excellent colloidal stability (100%). There was no significantly difference of preference scores at different level of stabilized-Calcium Carbonate added which indicated that Calcium enrichment using Calcium Carbonate did not affect consumer preference to the soy-red rice milk. The preference scores were in a range of 3.9 and 4.7, which is categorized as likely enough. Calcium content which determined by using a flame photometer, showed that calcium content of Ca-enriched soy-red rice milk at stabilized-Calcium Carbonate 0.5% w/v level was 133 mg/100 mL, comparable to calcium content of cow's milk which in a range of 125-150 mg/100 mL.

Keywords: *physicochemical, sensory, calcium, soy milk, red rice*

1. Introduction

Currently, soy milk is one of non-dairy milks which increasing the popularity as an alternative to cow's milk. Soy milk contains no lactose and cholesterol. It may reduce the risk of heart disease and some cancers because it contains isoflavones [1, 2]. The combination of soy milk with other materials have been developed especially combination with cereals such as combination of soy and corn; and soy and red rice to produce a nutritious soy-corn milk and soy-red rice milk [1, 3, 4, 5, 6]. Legume-cereal combination can produce non-dairy product with balance amino acid composition than legume or cereal separately. Soy-corn milk, which using yellow corn, is rich in carotenoids which is good for combating vitamin A deficiency. While the soy-red rice milk is rich in anthocyanin, fibers and vitamin B contents. Ratio of soybean and red rice of 1:1 was the optimum combination to produce soy-red rice milk with the best physicochemical and sensorial characteristics [6].

Soy-red rice milk is poor of calcium i.e. about 8 mg/100 ml, extremely lower than cow's milk i.e. 125-150 mg/100 ml [7]. Calcium is an essential nutrient because it is important for health bones and teeth; an important cell marker with respect to metabolic regulation and the transport of metabolites from one compartment to another or from one cell to the bloodstream; involved in the structure of the muscular system and controls essential processes like muscle contraction (locomotor system, heart-beat), blood clothing, activity of brain cells and cell growth [8, 9]. Several concerns have been raised regarding the use of calcium-fortified foods and beverages [10].

Calcium enrichment of food and beverage products can be done by addition a calcium source compound, such as Calcium Chloride, Calcium Carbonate, Dicalcium Sacharate, Calcium Lactate, Calcium Gluconate and Calcium Lactogluconate into the product [7, 11]. Each type of calcium source compounds

has certain calcium content, solubility properties, sensorial characteristics and bioavailability. Calcium enrichment of protein containing beverages often caused problems of instability to heat and during storage which due to the interaction between calcium ions with protein. Study on the effect of calcium addition on soybean protein isolates showed that amounts of calcium (1.23–5.0 mg/g protein) induced the formation of α,α′ soluble aggregates, whereas large amounts (5.0–9.73 mg/g protein) induced the selective insolubilization of the glycinin fraction. A decrease in the surface hydrophobicity of proteins with increasing calcium content was also observed [12].

This research objective was to study the physicochemical and sensory characteristics of Ca-enriched soy-red rice milk.

2. Materials and methods

Materials

Red rice, soybean and cane sugar were purchased from local market in Surabaya, Indonesia. Stabilized-Calcium Carbonate was obtained from PURAC Asia Pacific, Singapore. Chemicals for analysis included distilled water, Lanthanum Chloride (Merck) and standard of calcium (BWB Technology, UK) were obtained and purchased from local supplier.

Processing of soy-red rice milk

Processing of soy-red rice milk was carried out according to Stephanie (2007) through the following steps: soybean and red rice were washed to remove physical contaminants; then soaked the soybean and red-rice for 4 hours and 10 hours, respectively, in separate containers; soybean seed coat were removed; soybean and red rice were washed to remove soybean seed coat and contaminants; then drained until no water dripping. Red rice and soybeans were weighed with a total weight of 250 g with the ratio of soybean: rice red = 1:1. Red rice and soybean were mixed and crushed in a blender by adding water with ratio of soy-red rice : water = 1: 10, then filtered using filter cloth. The filtrate was added by cane sugar and stabilized calcium carbonate at different level i.e. 0.1; 0.2; 0.3; 0.4 and 0.5% w/v. After that the mixture was pasteurized at 85^{o}C for 15 minutes then cooled and put in plastic bottles. Products obtained were analyzed for physicochemical characteristics i.e viscosity, colloidal stability and calcium content; and sensory characteristic i.e preference of color, taste and aroma. The experiment and analysis were conducted in three replicates.

Viscosity, pH and colloidal stability measurement

The viscosity was measured using viscometer (Brookfield model DV-E). 250 mL of soy-red rice milk in beaker glass was measured the viscosity using spindle 1 with minimum accuracy of 95%. The colloidal stability of soy-red rice milk was measured according to Srianta et al. (2010) with modification. 10 mL of soy-red rice milk samples were placed in graduated tubes held in racks in the refrigerator undisturbed at 4^{o}C for 3 days. Changes in colloidal stability were indicated by separation into two layers. Level of visible line of demarcation between the settled and remaining portion of the milk solution was measured in daily during 3 days of storage.

Sensory evaluation

The sensory evaluation of the soy-red rice milk were done by 90 panelists who are familiar with the soymilk. Hedonic method was used with scale of 1 represent dislike extremely to 9 represent like extremely. The panelists were requested to evaluate the taste, aroma and

color of the soy-red rice milk. The test was conducted in sensory evaluation room.

Determination of calcium content

4 mL of sample was digested in digestion flask containing concentrated Hydrochloric Acid and Nitric Acid, then 5 mL of Hydrochloric Acid 4 N was added into the solution, adjusted until 100 mL with distilled water in measuring flask. Calcium content was measured by using a flame photometer (BWB XP, UK) after the solution was added by 2 mL of Lanthanum Chloride 10%. Calcium solution (BWB Technology, UK) was used as calcium standard.

Statistical analysis

Data analysis was done by analysis of variance (ANOVA) with $\alpha = 5\%$ and followed by the Duncan's Multiple Range Test (DMRT) with $\alpha = 5\%$.

3. Results and discussions

Physicochemical characteristics

Table 1 show the physicochemical characteristics of soy-red rice milk at different level of stabilized-Calcium Carbonate. Calcium content was higher as higher stabilized-Calcium Carbonate level added. Range of calcium contents of Ca-enriched soy-red rice milk were between 41.78 and 133.00 mg/100 mL. Soy-red rice milk without addition of stabilized-Calcium Carbonate shows very low calcium content of 7.75 mg/100 mL, which indicated low calcium contents of soybean and red rice which was used as raw materials. Addition of 0.5% w/v level of stabilized-Calcium Carbonate produce soy-red rice milk with calcium content comparable to cow milk, i.e. 125-150 mg/100 mL [7].

Higher stabilized-Calcium Carbonate added higher viscosity of Ca-enriched soy-red rice milk. The viscosity of aqueous system depends on water binding and holding capacities of components of the system. Viscosity of Ca-enriched soy-red rice milk was higher as higher level of stabilized-Calcium Carbonate added. This indicated that free water in the system was bound and entrapped by gellan gum in the stabilized-Calcium Carbonate added.

Scilingo and Anon (2004) reported that calcium addition up to 5 mg ion/g isolate protein did not significantly affect solubility, however from this calcium content upward, solubility of soy protein isolate decreased with calcium increase. Calcium induced the selective insolubilization of the glycinin fraction. However, this phenomenon was not occurred in the Ca-enriched soy-red rice milk. Colloidal stability of Ca-enriched soy-red rice milk was 100% (excellent) at all stabilized-Calcium Carbonate addition level. This might be due to the presence of gellan gum in the stabilized calcium carbonate, which form network by binding and entrapping free water and solid substances in the system.

Sensory characteristics

Table 2 show Sensory characteristics of soy-red rice milk at different level of stabilized-Calcium Carbonate. Calcium enrichment using stabilized-Calcium Carbonate at all concentration did not affect the preference scores of color, taste and aroma. Ca-enriched soy-red rice milk preference scores was in a range of likely enough. Soy-red rice milk is light pink which indicate anthocyanins presence in the product, as also reported by Stephanie (2007). Addition of stabilized-Calcium Carbonate did not affect the color of soy-red rice milk hence panelists provide the same score for all level of stabilized-Calcium Carbonate added. According to

Zhao et al. (2005), Calcium Carbonate has a chalky taste. However, chalky taste was not detected in Ca-enriched soy-red rice milk, even of the highest level of stabilized-Calcium Carbonate addition. This probably related to the sweetness of the product from cane sugar which was added into the product at 7% w/v. Aroma of the product was combination of red rice and soybean. Addition of stabilized-Calcium Carbonate did not affected aroma of the soy-red rice milk. This might be due to stabilized calcium carbonate is odorless, as stated by Camara-Martos and Amaro-Lopez (2002).

Table 1. *Physicochemical characteristics of soy-red rice milk at different level of stabilized-Calcium Carbonate*

Stabilized-Calcium Carbonate (%)	Calcium content (mg/100 ml)	Viscosity (cP)	Colloidal stability (%)
0	7.75^{a}	100^{a}	100
0.1	41.78^{b}	125^{b}	100
0.2	61.04^{c}	175^{c}	100
0.3	79.89^{d}	200^{d}	100
0.4	101.46^{e}	250^{e}	100
0.5	133.00^{f}	300^{f}	100

Note: different character indicated a significantly difference at $\alpha = 5\%$

Table 2. *Sensory characteristics of soy-red rice milk at different level of stabilized calcium carbonate*

Stabilized-Calcium Carbonate (%)	Preference score		
	color	taste	aroma
0	4.1^{a}	4.2^{a}	4.2^{a}
0.1	4.2^{a}	4.2^{a}	4.0^{a}
0.2	4.2^{a}	3.9^{a}	4.0^{a}
0.3	4.2^{a}	4.0^{a}	3.9^{a}
0.4	4.0^{a}	4.7^{a}	4.0^{a}
0.5	4.2^{a}	3.9^{a}	4.1^{a}

Note: different character indicated a significantly difference at $\alpha = 5\%$

4. Conclusions

Viscosity and calcium content of Ca-enriched soy-red rice milk were affected by stabilized-Calcium Carbonate level added. Ca-enriched soy-red rice milk has an excellent colloidal stability (100%) at all stabilized-Calcium Carbonate level.

Sensory characteristics of Ca-enriched soy-red rice milk were not significantly different at difference level of stabilized-Calcium Carbonate added. Further research on calcium bioavailability of soy-red rice milk is recommended.

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GROWTH OF MEDIAL TIBIAL PLATEAU (MTP) CARTILAGE DUE TO CONSUMPTION OF DIFFERENT AMOUNTS OF SOYA BEAN MEAL AT DIFFERENT AGES IN FEMALE MICE

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ABSTRACT

The incidence of osteoarthritis, one of most prevalent articular diseases, in particular in aged ones, has led to study the effect of different amounts of soya bean meal on the growth of Medial Tibial Plateau Cartilage (MTP) which has an important role in knee joint.

In this study, 50 immature female mice, were divided into 5 groups and while using different diets for 6 months. At group A, the mice were fed by diet with full protein containing 20% of soya bean meal. At group B, the mice used diet with full protein containing 40% of soya bean meal. Another groups the animals were fed by protein deficit diet for 3 months and in 2nd three-month period, the mice of group C used diet as same as diet of group A and, mice of group D used diet as same as diet of group B and finally the mice of group E consumed full protein diet without soya bean meal. After 6 months, to determine the rate of serum alkaline phosphate, and the size of Medial Tibial Plateau Cartilage, following tissue preparation, H&E Staining and Toluidine blue (TB) were used .

The results showed that there are significant differences between groups used different amounts of soya bean meal and other groups on the size of MTP cartilage, concentration of intercellular matrix and the rate of ALP ($P<0.001$). To evaluate the data, Kruskal–Wallis Analysis, One-way Variance Analysis and Independent Sample were used.

From this study it is implied that in case of using soya bean meal in diet, it may be effective on the growth of Cartilage, in knee joint. As well, it's different rate will have different effects on cartilage growth.

Keywords: *growth , Toluidine blue, alkaline phosphate ,diet*

1. Introduction

Osteoarthritis or Joint Degenerative Disease is one of the most ancient recognized diseases which have been found at the skeletons of Frozen Era. Generally, It occurs in body weight tolerating joints such as knees. In this disease, the superficial layer of cartilage is degenerated and destroyed (1&2). Articular cartilage is made from chondrocytes and extra cellular matrix composed of collagen in a bed of hard hydrated gel, proteoglycane and different glycoproteins which are continuously made and substituted. Most important part of cartilage is the large amount of cartilage-bound water which has been attached to the negative charges of glycose aminoglycanes and performs as a shock absorber or a mechanical spring (3).

In case of disequilibrium between synthesis and catabolism of macromolecules of extracellular matrix of cartilaginous tissue, it leads to deterioration of biochemical contents of extracellular matrix and the cartilage will be demolished. (4).

Nowadays, there is a noteworthy idea on the use of vegetative materials to improve osteoarthritis (5). Bromelian, derived from Pineapple, demonstrates anti-inflammatory and analgesic properties in osteoarthritis (6).

Other effective plants in control of osteoarthritis are soya and avacado (7).

Different in vitro studies regarding the effects of unsaponified avacado and soya on articular cartilage have showed a 2:1 unit of Soya/Avacado mixture was more effective than separately unsaponified soya or avacado on the improvement of osteoarthritis (8).

One other study has been done on the effect of unsaponified avacado and soya on the increase of articular cartilage thickness after knee joint meniscectomy (4).

In other study, it was shown that unsaponified avacado and soya could synthesize the collagen by chondrocytes stimulation and prevents cartilage destruction and lead to its improvement (8). There are a lot of researches on determining the compounds in unsaponified avacado and soya (ASU) leading to the improvement of osteoarthritis. In this regard, it is pointed to biologically active compounds classified as unsaponified lipids (9). Early studies have shown that Phytosterols in general and Beta- sitosterols in particular are considered as anti-inflammatory agents with antioxidant and analgesic activities (10).Anti-inflammatory property of sterols, as vegetative sources, has been found in the tests on the animals (11). As well, there are other studies on the increase of serum alkaline phosphate in groups using soya bean meal (12).

The aims of this study are:

1-If the consumption of soya bean meal before maturation may significantly effect on the growth of MTP cartilage?

2-If the consumption of different amounts of soya bean meal has different effects on the growth of cartilage? Or in other words, if the cartage growth due to soya consumption is related to the amount and time of its consumption?

2. Materials and methods

Materials

In this study, 50 immature female mice, BALB/c race, 12-15 gram were purchased from Iran Pasture Institute. The mice were kept at Animals Room with controlled temperature of 23+- C and light/dark periods of 12 hours. Food and water were ad libitum. To do the tests, the mice were divided in 5 groups and were fed by the following diet for 6 months:

Group A: the mice were fed by the diet with full protein including corn powder, colza bean meal, sunflower bean, shell powder, Di-calcium phosphate (DCP), mineral supplement, vitamin supplement, salt as well as soya bean meal which had provided %20 of required protein of the mice.

Group B: The diet of this group was as the same as group A, with difference that soya bean meal provided 40% of required protein.

Group C: In this group, the mice were fed firstly by protein deficit diet for 3 months and then used the diet of group A for the 2nd three-month period.

Group D: The mice of this group at 1st three-month period was fed by deficit protein diet and then by diet of group B at 2nd three-month period.

Group E: The mice of this group were firstly fed by deficit protein diet for 3 months, and at 2nd three-month period they used full protein diet without soya bean meal.

After 6 months, the mice were anesthetized and bleed were done from their hearts. Blood sera of all groups were collected and sent to the laboratory (12).

To study the size of MTP cartilage, the posterior limbs were detached. After trimming and separation of soft tissues, the samples were put in 10% formalin.

To prepare the cartilaginous tissues, by using the Kristiansen's Solution, the tissue decalcification was done as follows:

The medial tibial plateaus were decalcified for 72 hours in a 1:1 mixture of 8 N Formic acid and 1 N sodium format rinsed for 24 hours with cold tap water (13). After samples dehydration by alcohol and moulding by paraffin, 5 micrometer thick sagital sections of MTP from lateral to medial sides were prepared. Some of the sections were stained by Toluidine Blue (TB) and some by hematoxylin and eosin stain (H&E).

For histomorphometric evaluation of cartilage, the sections were photographed directly and examined using stereo microscope at X 400 high power equipped with Microsoft System software connecting to PC (14).

Photographed sections were evaluated from the view point of the size of medial part of MTP cartilage.

TB stained sections were used to determine the concentration of intercellular matrix. Stain density which is related to the rate of proteoglycan of extra cellular matrix, was assigned as evaluation criteria (15).

In case of thickness of cartilage of medial part of MTP and the rate of blood serum alkaline phosphate, one way variance (ANOVA) was used and to determine the stain density of extracellular matrix, Kruskal–Wallis Test was used. The rate of evaluation for data, was assigned $P<0.001$.

3. Results and discussions

Effects of soya bean meal on medial part of MTP cartilage

The results of the survey on the effects of soya bean meal on the thickness of medial part of MTP cartilage indicate the significant difference between the group of rats which used the diet with full protein (20% of diet was soya bean) and the groups which consumed deficit protein diet at their 1st three-month life after suckling period and then used included soya bean meal diet at 2nd three-month, that is, they used soya bean meal during shorter period and/or they used full protein diet at 2nd three-month, $P<0.001$ (Table 1).

As well, the results in group of mice which used the diet with full protein (40% of diet was soya bean) for 6 months in comparison with the groups of mice which used full protein in their diet and/or the groups used soya bean meal in shorter period, showed significant difference ($P<0.001$).

In groups used diet of soya bean meal (20% protein) for 6 months, the thickness of MTP cartilage showed dominance to the group used diet of soya bean meal (40% protein) of for 6 months, in other words, long term consumption of diet of soya bean meal (20% protein) is dominant to cartilage growth than the long term consumption of soya bean meal (40% protein) (table 2).

Effects of soya bean meal on concentration of intercellular matrix

In group used diet of soya bean meal (20% protein) for 6 months in comparison with those groups used full protein diet or consumed soya bean meal in shorter time, the concentration of intercellular matrix shows significant difference (Table 1). As well, in group used diet of soya bean meal (40% protein) for 6 months, there was significant increase compared with those groups not using soya

bean meal in their diet or used soya bean meal in shorter period (Table 2). Also, concentration of intercellular matrix in group used diet of soya bean meal (20% protein) in long term is dominant to the group used diet of soya bean meal (40% protein) in long term (Tables 1 and 2). Finally, the results showed that duration elongation and the rate of soya bean meal in diet are two main intervening criteria to increase of concentration of intercellular matrix.

Effect of soya bean meal on the rate of serum alkaline phosphatase

There was found that the serum alkaline phosphatase shows significant increase in the group used diet of soya bean meal (20% protein) for 6 months in comparison with other groups, $P<0.01$. As well, Serum Alkaline Phosphatase of has significant difference in the that group used diet of soya bean meal (40% protein) for 6 months in comparison with other groups, $P<0.01$. Also, in group used long term soya bean meal (20% protein), there was significant difference with the group used soya bean meal (40% protein) bean in long term, $P<0.01$.

Table 1. *Comparison of the group used diet with full protein (20 % soya bean meal) for 6 months in comparison with other groups*

Group **Index (Criteria)**	**Feed of Group A with Group E**	**Feed of Group A with Group C**	**Feed of Group A with Group D**
Thickness of Medial Part of MTP Cartilage	227.27±49.11 a 103.24±17.76 b	227.27±49.11 a 148.84±14.05 c	227.27±49.11 a 209.86±56.05 a
Concentration of Intercellular Matrix	3.1±0.96 a 1.66±0.51 b	3.1±0.96 a 2.66±0.72 c	3.1±0.96 a 1.72±0.25 b
Alkaline Phosphatase (ALP)	62.74±0.8774 a 30.58+-1.014 b	62.74±0.8774 a 34.15+±2.262 b	62.74±0.8774 a 41.63±1.761 b

Group A: Diet with full protein (20% soya bean meal)
Group B: Diet with full protein (40% soya bean meal)
Group C: Protein deficit diet at 1st three-months and diet including 20% soya bean meal at 2nd three-months
Group D: Protein deficit diet at 1st three-months and diet including 40% soya bean meal at 2nd three-months
Group E: Protein deficit diet at 1st three-months and full protein diet at 2nd three-months
Mean± SE,unequal letter(s) in each data indicate significant difference at the level of p<0/001

Table 2. *Comparison of the group used diet with full protein (40% soya bean meal) for 6 months in comparison with other groups*

Group **Index (Criteria)**	**Feed of Group B with Group E**	**Feed of Group B with Group C**	**Feed of Group B with Group D**
Thickness of Medial Part of MTP Cartilage	215.49±22.73 A 103.24±17.76 b	215.49±22.73 a 148.84±14.05 c	215.49±22.73 a 209.86±56.05 a
Concentration of Intercellular Matrix	2.72±0.99 a 1.66±0.51 b	2.72±0.99 a 2.66±0.72 b	2.72±0.99 a 1.72±0.25 b
Alkaline Phosphatase (ALP)	52.69±1.314 a 30.58±1.014 b	52.69±1.314 a 34.15±2.262 b	52.69±1.314 a 41.63±1.761 a

Group A: Diet with full protein (20% soya bean meal)
Group B: Diet with full protein (40% soya bean meal)
Group C: Protein deficit diet at 1st three-months and diet including 20% soya bean meal at 2nd three-months
Group D: Protein deficit diet at 1st three-months and diet including 40% soya bean meal at 2nd three-months
Group E: Protein deficit diet at 1st three-months and full protein diet at 2nd three-months
Mean± SE,unequal letter(s) in each data indicate significant difference at the level of p<0/001

4. Conclusions

Osteoarthritis or destruction (deterioration) of articular cartilage is one of more prevalent articular diseases in middle age in which knee joint shows most traces of osteoarthritis (1). Drugs to cure osteoarthritis, used almost as anti-inflammatory and analgesic than for treatment, so, nowadays a lot of researches have been performed for repair of destructed (deteriorated) cartilage and/or for sustainability, preservation and freshness of cartilage and prevention of its destruction. So, the approach to herbal drugs and their suitable effects on repair of cartilage and compensation of lost parts have attracted a lot of attention. Amid plant materials, it may be mentioned avacado and soya bean meal. Researches on the effects of edible soya on the cartilage of knee joint have showed that in the cartilage of meniscectomized animal, the thickness of non- calcified cartilaginous area of Lateral Femur Chondral and Medial Femur Chondral has been increased by the use of edible soya bean meal (4). Other researches have been done in relation to consumption of unsaponified avakado/soya bean meal as a suitable treatment of osteoarthritis (14 and 4).

At the present research it was found significant increase in the thickness of tibial cartilage in those groups used soya included diet for 6 months in comparison with the groups not using soya bean meal in their diet or used soya bean meal in shorter period. As well, in this study, it was shown that in group used soya bean meal for a longer period but in lesser extent, the thickness of tibial cartilage is more than the group used soya bean meal for a longer period but in more extent.

In other study on the role of ASU on the cartilage, it was shown that this material has anti-destructive property and leads to the increase of repair and

renovation of articular cartilage. For example, anti-catabolic (anti- destructive) activity of ASU, directly leads to the stimulation of collagen producing and proteoglycans and therefore to change the growth factor of beta synthesis (16). In recent study, it was shown that the concentration of intercellular matrix whose most part have been constituted from collagen and proteoglycans, has been significantly increased in groups used soya bean meal included diet in comparison with not using soya bean meal in their diets or used it in a shorter period.

Some other studies were done on the etiology of cartilage destruction in osteoarthritis. These studies showed that the Interlokin-1 increase in osteoarthritis is one of the important factors in cartilage destruction and due to the increase of this material, destruction of articular cartilage commences. These researches which were done in vitro, showed that in case of increase of IL-1, metalloproteasases, nitric oxide and synthesis of eicosanoid will be activated and leads to destruction of cartilage and tissue inflammation (17).

Now, regarding the reports on the effects of avacado and soya bean meal on the treatment of osteoarthritis and also the present study which is précising the cartilage growth following consumption of soya bean meal, so, it is important to verify the role of these plant materials on the cartilage and how these could be effective on the growth of cartilage.

Different studies have shown that ASU may activate the synthesis of aggrecan and prevent the stromelysin activity of chondrocytes afflicted by osteoarthritis (18). On the other hand, the reason why avacdo/soya bean meal may prevent cartilage destruction and lead to its grow, is that on the study of the effect of avocado/soya bean meal extract (piascledine) on the collagenolytic activity of cultured rabbit articular chondrocytes and human rheumatoid synovial cells, the result suggest a potential role for piascledine to limit the deleterious effect of interleukine-1 in osteoarticular diseases by reducing the capacity of this cytokine to stimulate collagenase production by synoviocytes and chondrocytes (19).

Other result from different researches on ASU and its role on cartilage growth and non-destruction of cartilage, is the effect of this material on fibroblasts. It seems fibroblasts also response to ASU so that MMP2 and MMp3 metalloproteasases are controlled by low doses of ASU while tissue preventers of metalloproteasases are increased in upper doses (18). In this study, it was found that if soya bean is used at the rate of 20% for long term in diet, it has more effect on the thickness of tibial cartilage and concentration of intercellular matrix in comparison with the long term use of diet including 40% soya bean meal.

Following the effect of ASU in treatment of osteoarthritis, researches tried to analyze the components of ASU. At the result of these researches, they attributed the effectiveness of ASU in treatment of osteoarthritis to the presence of phytosterols in general and in particular to beta cytosols and recognized them as anti-inflammatory agents having anti-oxidant and analgesic properties (10). On the other hand, regarding the importance of ASU and/ or soya bean meal on the growth of cartilage, they also found the components other than sterols including a lot of lipid soluble vitamins and fatty acids.

Nowadays, there have been done more studies on recognition of sterol compounds and showed that the rate of effect of ASU on treatment of osteoarthritis is dependent to the rate of sterol in ASU (20). In this study it was found that the thickness of Tibial cartilage and concentration of intercellular matrix depend to the rate of consumption of soya bean meal and the duration of its consumption.

Regarding the rate of alkaline phosphate in healthy articular cartilage and osteoarthritic articular cartilage, there have been done some experiments and close relation between active alkaline phosphate and the thickness of articular cartilage have been found (21 and 22). In this study, it was found the increase of serum alkaline phosphate in diets including soya bean meal in comparison with diets without soya bean meal or diets including soya bean meal which have been used in shorter period. On the other hand, the relation between the rate of alkaline phosphate and thickness of cartilage and also concentration of intercellular matrix were found. As well, different studies have shown that consumption of soya bean meal while increases the rate of serum alkaline phosphate, consequently calcium absorption in intestine is increasing (23). So, by increase of alkaline phosphate, number of chondrocytes and concentration of cartilage matrix on the area of hypertrophy of growth plate are increased following the soya bean consumption (24).

Regarding the above said points, it is implied that increased rate of alkaline phosphate may leads to increase of calcium absorption and results to increase of thickness of calcified area of cartilage and the thickness of cartilage. On study the results of the effect of soya bean meal on cartilage growth, it should be emphasized that the effect of soya bean meal on cartilage growth depends firstly on duration of its consumption, secondly to the rate of consumption and thirdly in which step of life it is used.

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EFFECT OF VITAMIN E ON STORAGE STABILITY OF SOUR CREAM BUTTER MADE FROM SHEEP MILK

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ABSTRACT

A study was carried out to determine the effect of vitamin E on storage stability of sheep butter. Sour cream butter was manufactured by inoculating the cream with 2% starter culture CSK G300. Vitamin E acetate was added in the butter at the rate of 60 I.U. per kilogram. The storage stability was determined for a period of four months. The results of schall oven test indicated per oxide value of 6.86 as compared to control which have high per oxide value 13.50. During storage of 120 days free fatty acids and para anisidine value increased. Control sample had para anisidine value of 13.55 and 11.90 in fortified butter. Free fatty acids increase was higher in control 0.14% and 0.11% in vitamin fortified butter after 120 days of storage. Vitamin E at the rate of 60 I.U can be used as antioxidant for good storage stability of sheep milk butter.

Keywords: *Vitamin E, butter, storage stability, oxidation*

1. Introduction

The nutritional value of sheep milk is very high as compared to cow milk. It contains two times more solids than cow milk and has higher levels of vitamin E and water soluble vitamins and minerals. It contains higher levels of butterfat, but actually lower in saturated fat than other types of milk and a good source of medium chain triglycerides which may play a role in reducing cholesterol levels. Sheep milk is higher in calcium than milk from the cow or goat and is a rich source of other important minerals such as zinc, magnesium, and phosphorus. Milk from sheep is also vitamin rich containing health amounts of vitamins A, D, and E. It's a particularly good source of the B vitamins including folic acid (Hardy, 2000). Butter is a popular dairy product in this part of world. The butter deteriorates by auto-oxidation of fat, which leads to flavor defects after 1 month to 2 years under the cold storage conditions, depending on the temperature of storage, quality of original milk and the proceeding of processes involved in butter manufacturing Walstra *et al*. (1999). Generation of off flavors is accompanied with the formation of hydroperoxides which are harmful to human health. The process of fat oxidation can be prevented by adding natural or synthetic antioxidant substances. However, it was found that the artificial antioxidants, like BHT (butylated hydroxytoluene) or BHA (butylated hydroxyanisole) are suspected to have carcinogenic activity. On the other hand, increasing sensitivity of consumers to synthetic ingredients as well as their increasing awareness about the effect of diet on their health contributed to the increasing trend to use natural additives for the stabilization of fat-containing foodstuffs. Vitamin E is a natural antioxidant present in appreciable quantities in vegetable oils; milk is also a good source of vitamin E. (Shiota *et al.*, 1999). Keeping in view the importance of butter, antioxidant potential of Vitamin E the present study was planed to find out the effect of vitamin E on chemical and sensory characteristic as well as storage stability of sour-cream butter from sheep milk.

2. Materials and methods

Sheep milk was obtained from Small Ruminants Training and Research Centre, University of Veterinary and Animal

Sciences, Lahore. Cream was separated with the help of hand operated cream separator. Sweet cream (40% fat) was pasteurized at 90 ^{0}C for 15 seconds and immediately cooled. The vitamin E (Vitamin E Acetate; BASF Germany) was obtained from United Industries Ltd. Faisalabad.

The cream was inoculated using 2% starter culture CSK G300 and ripened at 21 ^{0}C for 18 hours. The churning was carried out at 14 ^{0}C done in periodical, conical churn filled to 40% with cream. After the butter grains reached the diameter of 2-4 mm the butter milk was drained off and the grains washed three times with cold water 13 ^{0}C and hand worked to reach the proper dispersion of water droplets. The butter mass was then divided into two parts: one was mixed with 60 IU of vitamin E and the second was left without any additives (control butter). The butter samples were packed and stored at 4ºC. Directly after production the butter samples were subjected to the assessment of basic chemical composition and sensory evaluation. The water, non-fat solids and fat contents were assessed by the following the respective procedures as prescribed in AOAC (2000).

Free fatty acids and peroxide value (Meq/kg /kg of fat) of butter fat were conducted by following the respective methods as described in AOCS (1990). The iodine number was determined by using Wij's method. Schaal oven test was performed by following the method of (AOCS, 1990) with 20g butter oil samples, in triplicate 50mL beakers, containing 2% vitamin E stored in an oven at 63 $\pm$1^{0}C, for 120 hours. Absorptivities at 232, 270nm and peroxide values were analyzed in the butter. In the photooxidation chamber test, 20g of butter in triplicate 50mL beaker. Para anisidine value was determined by using double beam spectrophotometer according to the method of AOCS (1990).

Vitamin E was added at the rate of 60 IU and beakers were arranged in a rectangular box of 80cm length x 35cm height x 60cm width, provided with six of 20W fluorescent light bulbs, displayed 3 above and 3 under the samples, so that the light could equally reach all the samples. The chamber light intensity was 2,800 Lux. This assay was conducted for a period of 168 hours. The free fatty acids and peroxide value were determined according to the official methods (AOCS, 1990). The butter samples containing 60 IU vitamin E were stored at -10 ^{0}C. Peroxide value, iodine value, free fatty acids and para anisdine value was determined for a period of four months at the interval of one month.

Statistical Analysis:

The data was obtained by applying completely randomized design (CRD) and the outcome of the analyses was analyzed through analysis of variance technique Steel *et al.* (1997) using Cohort version 6.1 (Co-Stat 2003) to determine the level of significance. The separation of means or significant difference comparisons was made using Tuckey's HSD test.

3. Results and discussion

Chemical Composition: The results of chemical composition of vitamin E fortified butter are given in Table1. Addition of vitamin E non significantly decreased the fat and water content and increased the nonfat dry matter.This may be due to chemical composition of vitamin E. The results of this study are in close agreement with Spreer,(2005) stated that fat content in butter should be at least 84%

and moisture should not be more than 16%.

Table 1. *Composition of Butter*

Composition (%)	Type of Butter	
	Control	**Vitamin E**
Fat	84.13±0.74[a]	84.05±0.29[a]
Water	14.75±0.65[a]	14.88±0.33[a]
Non Fat Solids	1.12±0.29[a]	1.07±0.45[a]

Mean value of duplicate experiment, means with same superscript letter in rows are statistically non-significant by the test of Tuckey at 0.05 level of significance.

Table 2. *Peroxide value of Butter Containing Vitamin E in the Schaal Oven Test for 5 days at 63 ^0C*

Butter Types	Peroxide value (meq./kg)
Control	13.50[a]
Vitamin E (60 IU)	6.86[b]

Means of duplicate experiment, means with same superscript letter are statistically non-significant. by the test of Tuckey at 0.05 level of significance.

The results regarding peroxide value of butter fortified with vitamin E in the Schaal oven test are given in Table 2 which indicated that peroxide value of control and vitamiin E fortified butter increased during submission at high temperature for five days. Peroxide values of all the samples increased during submission to photoxidation chamber. This may be due to the reason that fat oxidation is catalyzed at higher temperatures in presence of light. Peroxide value of vitamin E fortified butter (6.86 meq/kg) was lower than control (13.50 meq/kg). This may be due to the natural antioxidant properties of vitamin E. The results of this study are in close accordance with the findings of Lejko *et al.* (2009). They added vitamin E to butter oil at three different concentrations and stored at 25 ^0C and studied the effect of addition for three months and concluded that higher concentrations of vitamin E were more use ful than lower concentrations. Butter oil containing 200 mg vitamin per kg showed minimum values of peroxides and para anisidine.

The addition of vitamin E has antioxidant effect on free fatty acids, iodine value, peroxide value and para anisidine value. Free fatty acids, peroxide value and para anisidinve value increased while iodine value decreased throughout the storage period of 120 days. The decrease in iodine value may be due to the oxidation of double bonds and free fatty acids content may be increased due to hydrolytic rancidity. Peroxide value may be increased due to the formation of peroxides and presence of pro oxidants in butter. The increase in free fatty acids, peroxide value, para anisidine value was lower in butter containing vitamin E this may be due to the antioxidant effect of

vitamin E. The results of this study are in line with the findings of Lejko *et al.* oxide value. Similar findings were (2009). They reported that addition of vitamin E inhibited free fatty acids and per reported by Ayar *et al.* (2004*).*

Table 3. *Effect of vitamin E on storage stability of butter*

	Control					Vitamin E fortified Butter (60 IU)				
Parameter	**0**	**30**	**60**	**90**	**120**	**0**	**30**	**60**	**90**	**120**
FFA (%)	0.05 ±0.03^{e}	0.06 ±0.03^{d}	0.08 ±0.05^{c}	0.11 ±0.02^{b}	0.14 ±0.02^{a}	0.05 ±0.01^{d}	0.05 ±0.02^{d}	0.07 ±0.03^{c}	0.10 ±0.02^{b}	0.11 ±0.05^{a}
IV Wij's	34.66 ±0.22^{a}	34.55 ±0.34^{a}	34.12 ±0.45^{a}	33.90 ±0.18^{b}	33.75 ±0.15^{b}	34.66 ±0.34^{a}	34.60 ±0.30^{a}	34.45 ±0.27^{a}	34.32 ±0.29^{a}	34.10 ±0.28^{a}
PV	0.15 ±0.09^{d}	0.25 ±0.10^{d}	0.45 ±0.18^{c}	0.80 ±0.36^{b}	1.35 ±0.56^{a}	0.15 ±0.06^{d}	0.20 ±0.09^{d}	0.35 ±0.11^{c}	0.64 ±0.32^{b}	0.90 ±0.42^{a}
AV	10.10 ±0.78^{c}	10.23 ±0.80^{c}	10.45 ±0.80^{c}	11.15 ±0.83^{b}	13.55 ±0.85^{a}	10.10 ±0.56^{b}	10.15 ±0.67^{b}	10.34 ±0.64^{b}	10.78 ±0.78^{b}	11.90 ±0.80^{a}

Means of duplicate experiment, means with same superscript letter in the same row are statistically non significant by the test of Tuckey at 0.05 level of significance.

FFA – Free Fatty Acids,**IV**- Iodine Value, **PV-** Peroxide Value,**AV-** Para Anisidine Value

4. Conclusions

The main objective of this research was to determine the effect of vitamin E on storage stability of sheep butter. It is evident from the results of schaal oven test and storage study for 120 days (Table 3) that vitamin E has significantly influenced the storage stability of sour cream sheep butter. The para aniside value, peroxide value and free fatty acid generation was momentously less than control. From the results of this study it can be concluded that vitamin E can be used as an effective antioxidant to prolong the storage stability of sheep butter.

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DEVELOPMENT OF PROTEIN ENRICHED EXTRUDED PRODUCT WITH ACID HYDROLYSED INVERT SYRUP

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ABSTRACT

Extruded foods such as noodles and vermicelli have recently attracted great attention as breakfast foods. This is because of the ease and convenience with which they could be served readily to all age groups in multitude varieties. Conventional extruded foods are made with refined flour and other basic ingredients. The present study aimed at developing an extruded product (Vermicilli) with ragi and soyflour sweetened with invert syrup obtained by acid hydrolysis. The resultant product was analysed for its nutrient content.

Keywords: *Extrusion, Vermicilli, Breakfast foods, invertsyrup.*

1. Introduction

Invert syrup is a carbohydrate sweetener that has come to use in processed foods only in the recent days. The increase in the use of this sweetener is attributed to its properties that help to improve the taste , texture and colour of any product into which it is incorporated. Apart from these properties it also helps to extend the shelf life of the products. During the production of invert syrup,acid hydrolysis takes place in which water is split into H+ and OH- ions and these ions become part of the new component. A pinch of cream of Tartar, or lime juice in the sugar water solution, could be used as a catalyst in the reaction that converts sugar to glucose and fructose.

Today snack foods are based to a large extent on extrusion technology. Extrusion is a process in which a food material is forced to flow, under one or more of a variety of conditions of mixing, heating and shear, through a die which is designed to form and/or puff-dry the ingredients. In the study that was conducted, invert syrup was prepared using hydrochloric acid by varying the temperature, quantity of acid, quantity of water and time. Due to the above variations "the degree of inversion" altered. The best syrup based on these parameters was selected for its application in extruded products.

The study was attempted with the following objectives are:

- To produce invert syrup with hydrochloric acid.
- To incorporate the invert syrup in extruded product.
- To enrich the extruded product with soyflour.

Overview of literature

Invert syrup (29)

Invert syrup is prepared by the hydrolysis of sucrose, glucose and fructose. This is achieved by subjecting a sucrose solution to acid and heat. Sugar syrup can be partly or fully inverted leaving part of the original sugar unchanged. These syrups are known as medium or partial invert sugar syrups. The term "invert" originates from the effect on the polarimeter instrument traditionally used to analyse sucrose solutions. Compared to pure sucrose, a mixture of glucose and fructose "inverts" the plane of polarized light, and so this is known as invert sugar. Applications of this include sugar confectionery, cakes and soft cookies, and bread rolls. Water activity is a key measure used to determine the shelf

life of many food products. If the water activity is too high, the food product will be more liable to microbial spoilage and so shorter shelf life. Invert syrup has a lower water activity than that of sucrose, and so can be used to give a greater preservative effect, so giving an increased shelf life.

Degree of inversion (35)

When a sugar solution is heated, a certain percentage of sucrose breaks down to form "invert" sugar.

$C_{12}\ H_{22}O_{11} + H_2O = C_6\ H_{12}O_6$ (glucose) + $C_6H_{12}O_6$ (fructose)

This invert sugar inhibits sucrose crystallization and increases the overall concentration of sugars in the mixture. This natural process of inversion, however, makes it difficult to accurately assess the degree of invert sugar that will be produced. As a way of controlling the amount of inversion, certain ingredients, such as cream of tartar or citric acid may be used. Such ingredients accelerates the breakdown of invert sugar, and thereby increases the overall percentage of invert sugar in the solution.

Extrusion cooking (14)

The extrusion cooking process was used for the production of breakfast cereals, as cereals flakes to replace the traditional process of making cereal flakes from maize grits. Confectionery products like liquorice, chewing gum, hard candies and cereal sugar products coated with chocolate, made on cereal based blends have been produced by extrusion cooking. Since the beginning of its use in food production , the process was evolved into one in which selection is made from many variables. The process variables in extrusion cooking are temperature, extent of shear, source of heat, pressure, shape of die and continuous extrusion versus cutting at die face. Process conditions are selected in consideration of the requirement of the specific ingredients and the desired product characteristic. The protein content of ingredients must be considered because of the effect of heat on protein. In some products the goal might be minimization of protein change. Starch is an ingredient that requires special consideration. Its gelatinization requires adequate time in the presence of moisture. Starch structures can be changed under high shear conditions, resulting in stickiness and selection of low shear, low-pressure conditions flavors minimum starch degradation.(Caldwell et al.,1990)

Enrichment with protein flours

Noodles prepared from the blend of durum semolina and aestivum flour with defatted soyflour exhibited harder texture as to those prepared from aestivum semolina-defatted soy flour blend. Higher water soluble nitrogen (WSN) and available lysine contents were observed in noodles prepared from the blends containing defatted soyflour. However WSN in noodles decreased considerably after cooking whereas lysine content remained the almost unaffected. Trypsin inhibitor as well as lipoxygenase activity were higher in uncooked noodles containing defatted soyflour and were inactivated completely after cooking .Wheat flour and soy-fortified biscuits prepared with standardized levels of ingredients were analysed for chemical composition,in vitro digestability and protein efficiency ratio(PER). Addition of 20% defatted soyflour in the recipe increased the protein,ash,crude fibre, calcium phosphorus, iron, sugar and available lysine content of biscuits. No trypsin inhibitor activity was found in soy biscuits but had marginally nonenzymatic browning than the control sample.

2. Materials and methods

The following materials and methods were followed for the preparation of invert syrup and incorporating it into an extruded product .

Materials

Pure granular sugar was used for the present study. Water of high quality and purity was used for syrup preparation and for the mixing of dough for the extruded product.

Hydrochloric acid (1 N) was selected for the preparation of invert syrup. Refined flour and ragi flour were used for the preparation of extruded products. Soy flour was also incorporated for enriching the extruded products.

Methodology

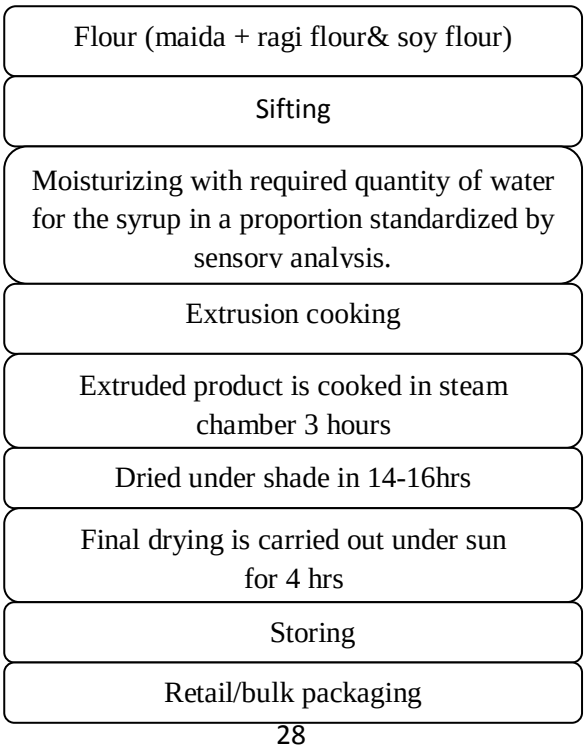
Preparation of invert syrup using hydrochloric acid

Invert syrup was prepared using hydrochloric acid by varying the temperature, quantity of acid, quantity of water and time.

Table 1. *Inversion of sugar using hydrochloric acid*

S. No.	Amount of acid (ml)	Amount of water (ml)	Amount of sugar (gms)	Time (mins)	Temp (°C)	Reducing sugar (%)	Total Soluble solids (°brix)
1	0.5	190	330	30	50	NIL	73.5
2	0.5	190	330	30	60	34.95	74
3	0.5	65	165	30	70	72.62	84
4	0.5	75	165	30	70	75.80	80
5	1.0	190	330	30	60	NIL	69.5

Preparation of an enriched extruded product with acid hydrolysed invert syrup



3. Results and discussions

Inversion of sugar using hydrochloric acid

On analysing the preparation of invert syrup using hydrochloric acid (Table 1) for a period of half an hour it was studied that at 50oC, there was absolutely no reaction. When the temperature was raised to 60^{o}C , it resulted in 34.95% reducing sugar content.

On further increasing the temperature to70^{o}C, there was a considerable increase in the percentage of reducing sugar to 72.62%.Since the syrup was highly viscous at this stage, the quantity of water was slightly increased at the same temperature resulting in 75.80% of reducing sugar and 20% moisture content which is 4% higher than the former with better fluidity.

The above analysis was done with 0.5 ml (0.15%) of hydrochloric acid. When the proportion of hydrochloric acid and sodium hydroxide are doubled at 60^{o}C it results in no breakdown of sucrose to glucose and fructose which implies that no inversion has taken place.

Figure 1 shows a graph plotted between reducing sugar (%) and temperature (^{0}C).

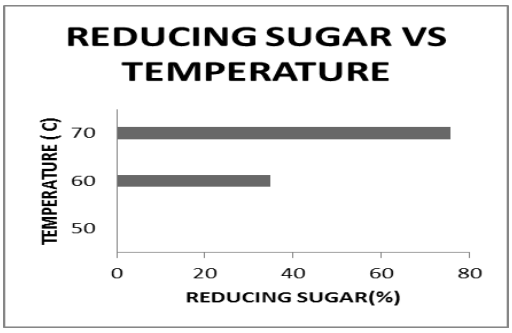


Figure 1

Incorporation of invert syrup in enriched extruded product

Extruded product was prepared with the accepted level of invert syrup using hydrochloric acid and the sample was enriched with soyflour.

Chemical analysis

When the enriched extruded product was chemically analysed , it was studied that there was an appreciable increase in the protein content of the extruded pr**oduct enriched with the soyflour.** Soy enriched product had the maximum amount of enrichment , but it had the minimum increase in the carbohydrates content.

Table 2. *Chemical analysis*

Flour used	Carbohydrate	Protein	Moisture content (% d.b.)
Soy	70.15	41.5	8.51

4. Conclusions

On the analysis of the different samples prepared using hydrochloric acid in varying concentrations, it has been found that two samples prepared at a temperature of 700C and at 0.5 ml of acid have undergone the inversion reaction to a greater extent leading to a reducing sugar percentage of 72.62% and 75.80%. Of these the latter is considered to be the best as it has better fluidity and has a higher degree of inversion. When the invert syrups were incorporated in extruded products, it was determined that 1 ltr of invert syrup produced using hydrochloric acid was the best. On enrichment of the extruded incorporated product with soy flour, it was found that the protein content of the product was highest when soyflour was used .Invert syrup prepared with different acids and incorporated in a variety of food products would thus pave way towards innovative new product development and set new trends in Indian food processing industry.

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STUDIES ON PREPARATION AND QUALITIES OF SWEET ORANGE BASED PRODUCTS

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ABSTRACT

The exploration of Sweet orange- Mosambi (Citrus sinensis) fruits for preparation of value added products such as squash, RTS, cordial from juice. The physico-chemical composition of orange juice was carried out. The yield of orange juice was 37.95%. The sweet orange fruit juice based products also studied for their physicochemical and microbial qualities in storage period under both ambient and refrigerated temperatures.

Keywords: *Sweet orange, Squash, RTS, Cordial, storage qualities*

1. Introduction

The sweet orange fruit is processed commercially in to various forms mainly juice, frozen concentrates, squash, RTS drinks, nectar, dry mixes, canned segments, juice blends, marmalades and other value added products like pectin and essential oil from peel, natural colors, candied peel, feed yeast etc. Fresh juice of sweet orange is an important nutritious product providing 45kcal, moderate quantity of vitamin C, potassium, bioflavonoid and folic acid and essential items of breakfast. It is refreshing, thirst quenching and energizing drink of breakfast. It is refreshing, thirst quenching and energizing drink that improves health and nutritional requirements. Citrus fruits and citrus juices have several beneficial health and nutritive properties.

Hence, in the light of above research fact, the present investigation was under taken with the following distinct objectives. Extraction of sweet orange juice and its utilization in various value added products viz squash, RTS and Cordial. Study the physicochemical and microbial qualities in storage period at ambient and refrigerated temperatures.

2. Materials and methods

Whole sweet orange (Mosambi) fruits were procured from local market of Parbhani . Proximate analysis was carried out for fruits as well as for finished products by AOAC (1990) method. Orange juice was extracted using Multi Fruit Juice Extracting Machine from Pilot plant of CFT, Parbhani.

Table 1. *Formulation of orange squash, orange RTS, orange cordial*

	Orange squash	Orange RTS	Orange cordial
Juice/pulp	250 ml	100 ml	250 ml
Water	750 ml	900 ml	750 ml
Citric acid	9 gm	2 gm	14 gm
Sugar	418 gm	72 gm	370 gm
Preservative (KMS)	350 ppm	---	350 ppm

Squash

Fruit juice squash consists essentially of strained juice containing moderate quantities of fruit pulp to which cane sugar is added for sweetening. The Orange juice was processed as Orange Squash according to the flow chart for the preparation of Orange squash is shown in Flow sheet 1.

Preparation of Squash

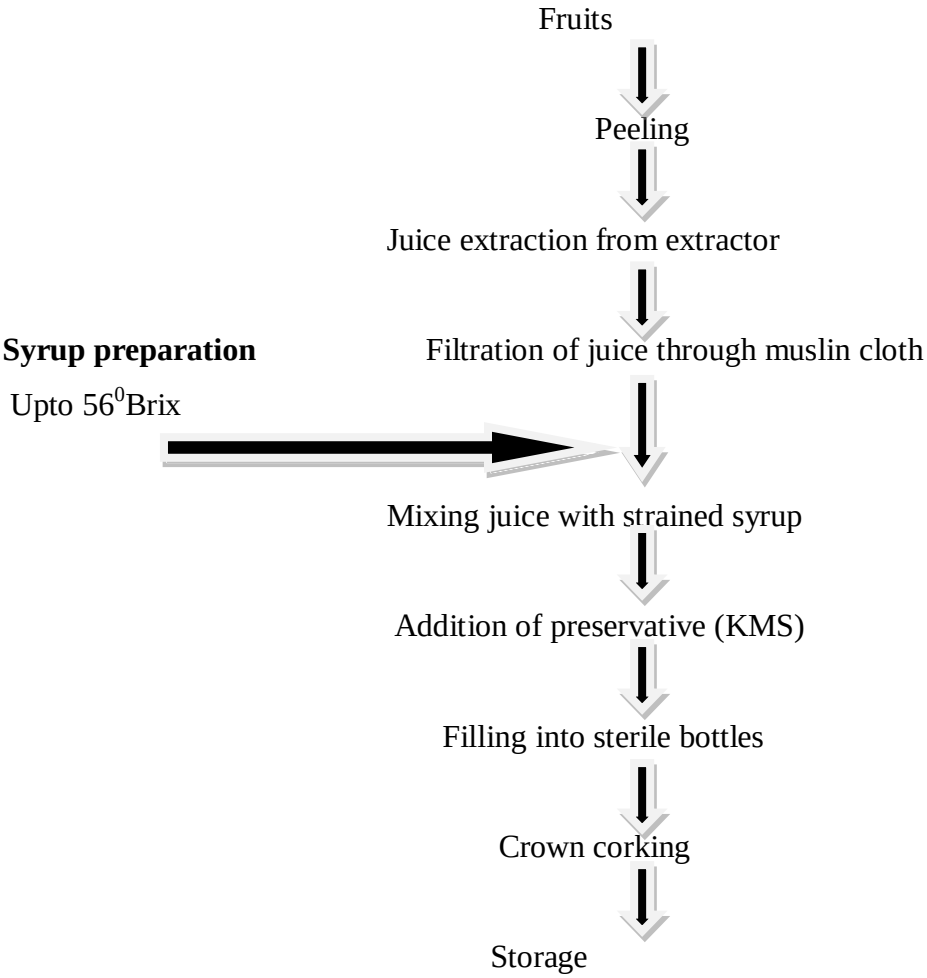

Figure 1. Process for Preparation of Orange Squash

Addition of ingredients

1500 ml of syrup (56^{0}Brix), citric acid (1%) and KMS @ 350ppm were added in 500 ml of Orange juice as per FPO specifications. All the ingredients were then mixed well.

Storage

The prepared squash was packed in glass bottles and was stored at ambient and refrigerated temperature.

RTS(Ready to Serve)

Fruit juice RTS is a fruit juice which is considerably altered in composition before packing. As the pack opened the juice is ready to drink. The Orange juice was processed as Orange RTS according to the flow chart for the

preparation of Orange RTS is shown in Flow sheet 2.

Preparation of RTS

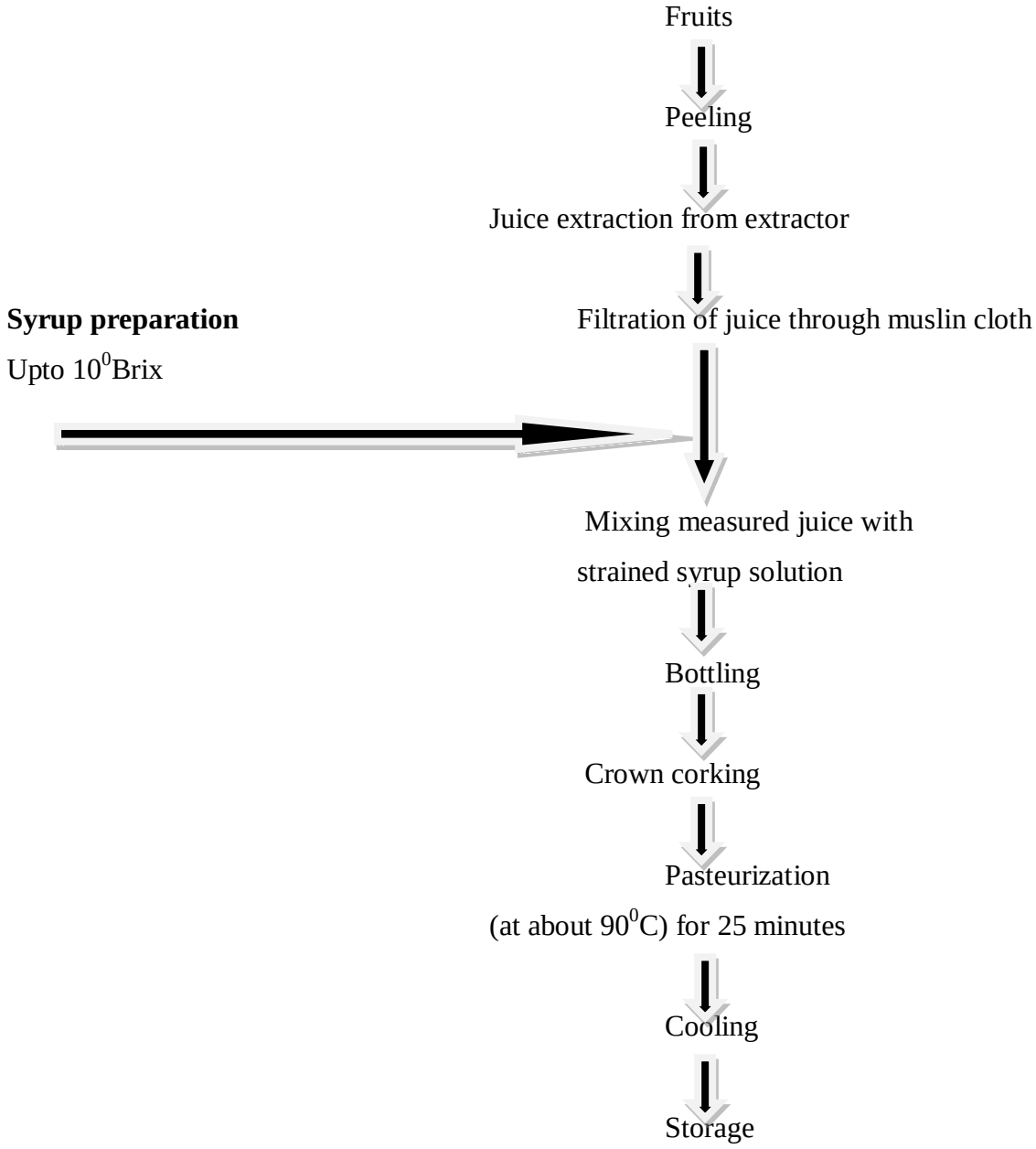

Figure 2. Process for Preparation of Orange RTS

Addition of ingredients 1800 ml of syrup (10^{0}Brix), citric acid (0.3%) were added in 200 ml of Orange juice as per FPO specifications. All the ingredients were then mixed well.

Storage

The prepared RTS was packed in glass bottles and was stored at both ambient and refrigeration temperatures.

Cordial is a sparkling, clear, sweetened fruit juice from which pulp and other impurities substances completely removed. The Orange juice was processed as Orange Cordial according to the flow

chart for the preparation of Orange Cordial is shown in Flows sheet 3

Preparation of Cordial

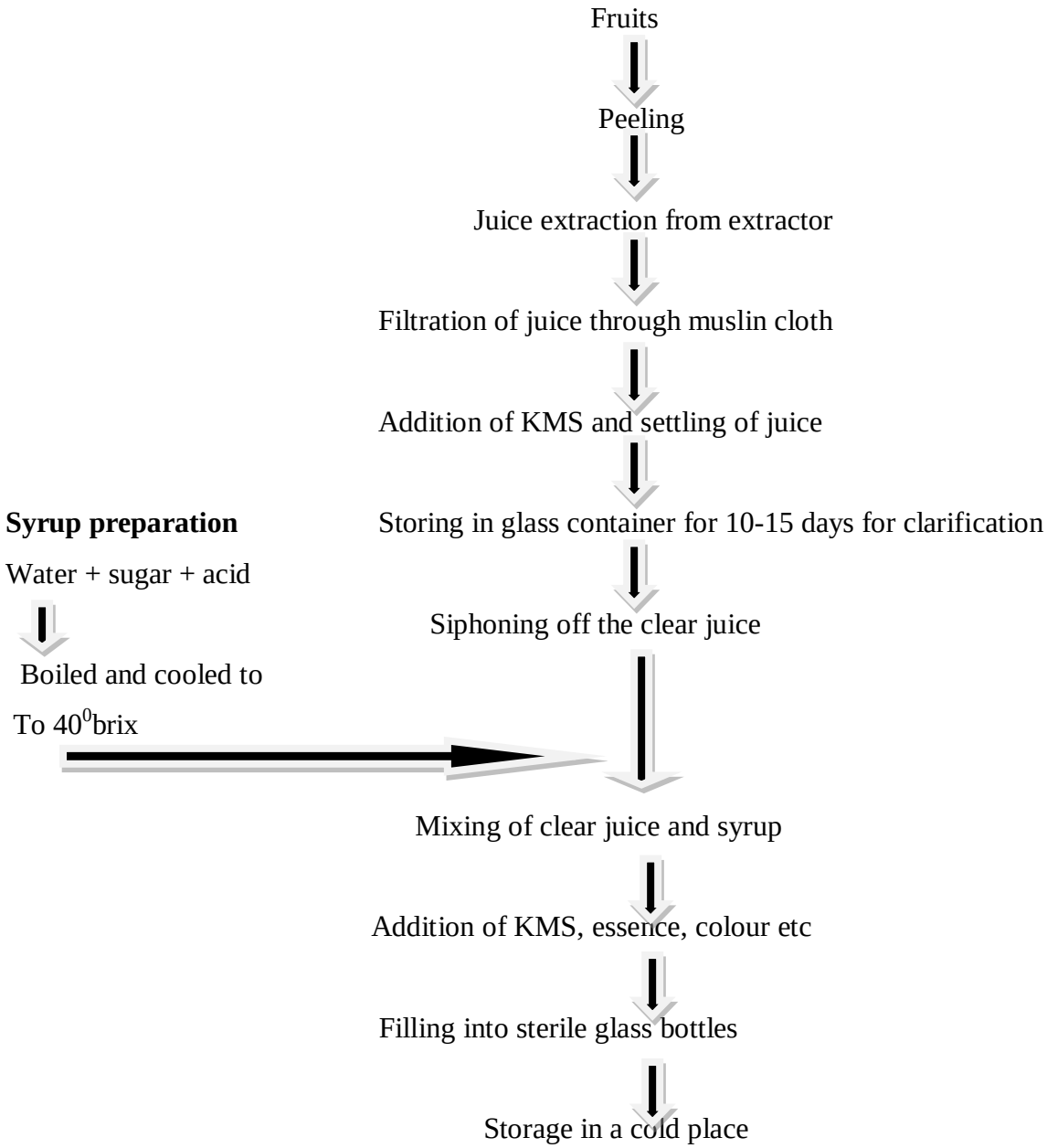

Figure 3. Process for Preparation of Orange Cordial

Addition of ingredients

1500 ml of syrup (40^{0}Brix), citric acid (1.5%) and KMS @ 350ppm were added in 500 of Orange juice as per FPO specifications. All the ingredients were then mixed well.

Storage The prepared Cordial was packed in glass bottles and was stored at ambient and refrigeration temperature.

Storage Studies of Orange Squash, Orange RTS and Orange Cordial

In order to undertake storage studies of the processed products, samples

were withdrawn from the three products kept at ambient (25-30^{0}C) and refrigerated (5-8^{0}C) temperature. All the products were stored for a period of two month. The effects of both temperatures on physicochemical, microbial and sensory quality were carried out.

Sensory Evaluation

Sensory evaluation was done on the 100 point Hedonic scale score (Ranganna, 1986) by the group of semi trained panel from Department of Food Chemistry and Nutrition, CFT, Parbhani which included faculty members. The evaluation was done according to the following attributes- Appearance, color, flavor, taste and over all acceptability. The obtained results were recorded in sensory sheet

Microbial quality

The microbial analysis of all preserved products- orange squash, orange RTS and orange cordial were carried out accordingly to Ranganna **(**1986**).** One ml of each of the sample was taken and to this 9ml of 0.5% saline was added and then further diluted to four folds. 1ml of each from appropriate dilution was plated in required medium and then incubation was carried out. In each count, after incubation, the average count of colonies present on petriplates were multiplied by dilution factor and expressed as cfu (colony forming unit)/ml of sample.

Total Plate Count:

The total plate count was taken according to the method described in Raganna (1986). Appropriate dilutions of each of the samples were transferred aseptically to sterile petriplates. Pour plating was done using nutrient agar (Hi media Laboratories Pvt.Ltd, Mumbai). Plates were then incubated at 37^{0}C for 24-48 hours respectively.

Yeast and Mold Count

Appropriate dilution of sample were pour plated in Potato Dextrose Agar (Hi Media Laboratories Pvt Ltd Mumbai) for 3-5 days at 30^{0}C

Coliform Count

Appropriate dilution of samples were made and transferred to sterile petriplates, pour plating was done using Mac conkey agar, pH 7.4 (Hi Media Laboratories, Pvt Ltd, Mumbai) Plates were incubated at 37^{0}C for 48hours.

3. Results and discussions

Yield and Physico-chemical Composition of Juice

The data obtained for the percent yield and physic-chemical composition of orange juice was recorded and presented in Table 1.

Table 2.*Percent yield and chemical composition of orange Juice*

Sr. No.	Parameters	Result
1	Yield (%)	37.95
2	pH	3.7
3	TSS ^{0}Brix	10
4	Total Acidity %	0.41
5	Moisture %	88.4
6	Protein %	0.6
7	Fat %	0.05
8	Carbohydrate %	10.5
9	Fiber %	0.12
10	Ash %	0.3
11	Ascorbic Acid (mg/100ml)	43

Table 2 shows percent yield and physico-chemical composition of sweet orange juice. The percent yield obtained was 37.95 percent. In respect of moisture, it is clear that the fruit contents 88.4 percent. With regards to protein, fat, carbohydrate, fiber and ash content in fruits juice was observed 0.6%, 0.05%, 10.5%, 0.12% and 0.3% respectively; pH, TSS and TA% was found to be (3,7), (10^0Brix), (0.41%) respectively.

Sweet orange squash

Physico-chemical properties of sweet orange squash

The squash produced by the methodology was kept at two different temperatures – ambient temperature (25^0C-30^0C) and refrigerated temperature (5^0C-8^0C). It was noted that the squash so prepared was found to be stable for a period of 2 months, the period till which the investigation was carried out. The values obtained for various parameters as analyzed are shown in Table 3 and Table 4. The pH of the squash kept at ambient temperature was found to range between 3.55-3.40 and between 3.55-3.49 for squash kept at refrigerated temperature. No significant difference was found in the pH of the squashes stored at different temperature. TSS (0Brix) ranged between 45.2-40.1^{0}Brix and 45.2-44^{0}Brix respectively. Some lowering in TSS (0Brix) values of squash stored at ambient temperature was observed. %TA (as citric acid) in squash stored at ambient temperature was found to reduce from 0.41 to 0.37, while in the squash stored at refrigeration temperature change in %TA (0.41-0.40%) was observed during the entire storage period. As expected the ascorbic acid content was found to slightly decrease during the storage in squashes at two different temperatures (14.5-11.25mg/100ml at ambient storage condition and 14.5-14.3mg/100ml at refrigerated storage conditions). Kansal (2003) had reported more or less similar results with respect to ascorbic acid content

So the changes in various parameters were found to be relatively higher in squashes stored at ambient temperature.

Table 3. *Effect of ambient temperature on physicochemical attributes of the orange squash*

Sr.No.	Days	pH	TSS(^{0}Brix)	% TA	AA (mg/100ml)
1	0	3.55	45.2	0.41	14.5
2	15	3.50	41.5	0.39	13.9
3	30	3.46	40.3	0.38	12.6
4	45	3.43	40.1	0.38	11.85
5	60	3.40	40.1	0.37	11.25

Table 4.*Effect of refrigerated temperature on physicochemical attributes of the orange squash*

Sr.No.	Days	pH	TSS(^{0}Brix)	% TA	AA (mg/100ml)
1	0	3.55	45.2	0.41	14.5
2	15	3.53	45	0.41	14.4
3	30	3.51	44	0.41	14.4
4	45	3.51	44	0.41	14.3
5	60	3.49	44	0.40	14.3

Microbial and sensory quality of sweet orange squash stored at ambient and refrigerated temperature

Microbiological examination of the sweet orange squash was carried out after 15 days of interval with respect to TPC, Yeast and Mould, and Coliform count. The data pertaining to microbial examination and sensory evaluation is shown in Table 5 and Table 6.

Table 5.*Sensory score and microbial quality of orange squash stored at ambient temperature*

Sr.No.	Days	Sensory score (overall acceptability)	TPC (cfu/ml)	Yeast and Mould count	Coliform count
1	0	8.65	ND	ND	ND
2	15	8.65	ND	ND	ND
3	30	8.60	ND	ND	ND
4	45	8.60	2 x 102	ND	ND
5	60	8.60	3 x 102	1 x 102	ND

Table 6.*Sensory score and microbial quality of orange squash stored at refrigerated temperature*

Sr.No.	Days	Sensory score (overall acceptability)	TPC (cfu/ml)	Yeast and Mould count	Coliform count
1	0	8.80	ND	ND	ND
2	15	8.80	ND	ND	ND
3	30	8.75	ND	ND	ND
4	45	8.75	ND	ND	ND
5	60	8.75	1 x 102	ND	ND

Microbiological examination of the sweet orange squash revealed that coliforms were found to be absent in the squashes throughout the period of investigation. The yeast and mold colony appeared after 60days and TPC after 30 days of storage in case of squash stored at ambient temperature as shown in Table 4. It is worth mentioning that till 45days there was no colony observed under any microbiological examination in sweet orange squash kept at refrigerated temperature (Table 5). Thus showing the product was prepared and stored under hygienic conditions.

Sensory evaluation

Organoleptic evaluation carried out on every 15th day of storage showed that the sweet orange squash thus prepared was quite acceptable till the end of the storage period. No observable differences in organoleptic properties were found among the sweet orange squashes at two different temperatures. Kamaljeet (2002) reported a shelf life of kinnow squash of over 3months. Thus the storage period obtained in current investigation is in consonance with those obtained by Kaur (2002).

Sweet orange RTS

Physico-chemical properties of sweet orange RTS

The RTS produced by the methodology was kept at two different temperatures – ambient temperature (25^0C-30^0C) and refrigerated temperature (5^0C-8^0C). It was noted that

the RTS so prepared was found to be stable for a period of 2months, the period till which the investigation was carried out. The values obtained for various parameters as analyzed are shown in Table 7 and Table 8.

Table 7.*Effect of ambient temperature on physicochemical attributes of the orange RTS*

Sr.No.	Days	pH	TSS(0Brix)	% TA	AA (mg/100ml)
1	0	4.12	11.8	0.39	17.1
2	15	4.11	11.51	0.39	16.5
3	30	4.10	11.32	0.39	15.8
4	45	4.10	11.24	0.39	15.1
5	60	4.09	11.03	0.39	14.8

Table 8.*Effect of refrigerated temperature on physicochemical attributes of the orange RTS*

Sr.No.	Days	pH	TSS(0Brix)	% TA	AA (mg/100ml)
1	0	4.12	11.8	0.39	17.1
2	15	4.11	11.51	0.39	17.0
3	30	4.10	11.32	0.39	16.9
4	45	4.10	11.24	0.39	16.9
5	60	4.09	11.03	0.39	16.8

The pH of the RTS kept at ambient temperature was found to range between 4.12-4.09 and between 4.12-4.09 for squash kept at refrigerated temperature. No significant difference was found in the pH of the RTS stored at different temperature. TSS (0Brix) ranged between 11.8-11.03^{0}Brix and 11.8-11.03^{0}Brix respectively. Some lowering in TSS (0Brix) values of RTS stored at ambient temperature was observed. %TA (as citric acid) in RTS stored at ambient temperature was found 0.39, while in the RTS stored at refrigeration temperature change in %TA (0.39) was observed unchanged during the entire storage period. As expected the ascorbic acid content was found to slightly decrease during the storage in RTS at two different temperatures (17.1-14.8mg/100ml at ambient storage condition and 17.1-16.8mg/100ml at refrigerated storage conditions).

Microbial and sensory quality of sweet orange RTS stored at ambient and refrigerated temperature

Microbiological examination of the sweet orange RTS was carried out after 15 days of interval with respect to TPC, Yeast and Mould, and Coliform count. The data pertaining to microbial examination and sensory evaluation is shown in Table 9 and Table 10.

Table 9.*Sensory score and microbial quality of orange RTS stored at ambient temperature*

Sr.No.	Days	Sensory score (overall acceptability)	TPC (cfu/ml)	Yeast and Mould count	Coliform count
1	0	8.65	ND	ND	ND
2	15	8.65	ND	ND	ND
3	30	8.60	ND	ND	ND
4	45	8.60	2 x 102	ND	ND
5	60	8.60	3 x 102	ND	ND

Table 10.*Sensory score and microbial quality of orange RTS stored at refrigerated temperature*

Sr.No.	Days	Sensory score (overall acceptability)	TPC (cfu/ml)	Yeast and Mould count	Coliform count
1	0	8.80	ND	ND	ND
2	15	8.80	ND	ND	ND
3	30	8.75	ND	ND	ND
4	45	8.75	ND	ND	ND
5	60	8.75	1 x 102	ND	ND

Microbiological examination of the sweet orange RTS revealed that coliforms and Yeast and Mould count were non detected in RTS throughout the period of investigation stored at refrigerated temperature. The yeast and mould count appeared at 60 days and TPC appeared at 45 days of storage in case of RTS stored at ambient temperature as shown in Table 8. It is worth mentioning that till 45 days there was no colony observed under any microbiological examination in sweet orange RTS kept at refrigerated temperature (Table 9). Thus showing the product was prepared and stored under hygienic conditions.

Sensory evaluation

Organoleptic evaluation carried out on every 15th day of storage showed that the sweet orange RTS thus prepared was quite acceptable till the end of the storage period. No observable differences in organoleptic properties were found among the sweet orange RTS at two different temperatures.

Sweet orange cordial

Physico-chemical properties of sweet orange cordial

The cordial produced by the methodology was kept at two different temperatures – ambient temperature (25^0C-30^0C) and refrigerated temperature (5^0C-8^0C). It was noted that the cordial so prepared was found to be stable for a period of 2 months, the period till which the investigation was carried out. The values obtained for various parameters as analyzed are shown in Table 11 and Table 12.

Table 11.*Effect of ambient temperature on physicochemical attributes of the orange cordial*

Sr.No.	Days	pH	TSS(0Brix)	% TA	AA (mg/100ml)
1	0	3.42	31.5	0.71	16.5
2	15	3.40	31.0	0.68	15.8
3	30	3.39	30.6	0.65	15.2
4	45	3.38	30.3	0.63	14.7
5	60	3.38	30.1	0.62	14.5

Table 12.*Effect of refrigerated temperature on physicochemical attributes of the orange cordial*

Sr.No.	Days	pH	TSS(0Brix)	% TA	AA (mg/100ml)
1	0	3.42	31.5	0.71	16.5
2	15	3.42	31.2	0.69	16.4
3	30	3.41	30.9	0.68	16.4
4	45	3.41	30.7	0.67	16.3
5	60	3.40	30.7	0.67	16.2

The pH of the cordial kept at ambient temperature was found to range between 3.42-3.38 and between 3.42-3.40 for cordial kept at refrigerated temperature. No significant difference was found in the pH of the cordials stored at different temperature. TSS (0Brix) ranged between 31.5-30.10Brix and 31.5-30.70Brix respectively. Some lowering in TSS (0Brix) values of cordial stored at ambient temperature was observed. %TA (as citric acid) in cordial stored at ambient temperature was found to reduce from 0.71 to 0.62, while in the cordial stored at refrigeration temperature change in %TA (0.71-0.67%) was observed during the entire storage period. As expected the ascorbic acid content was found to slightly decrease during the storage in cordials at two different temperatures (16.5-14.5mg/100ml at ambient storage condition and 16.5-16.2mg/100ml at refrigerated storage conditions).

Microbial and sensory quality of sweet orange cordial stored at ambient and refrigerated temperature:

Microbiological examination of the sweet orange cordial was carried out after 15 days of interval with respect to TPC, Yeast and Mould, and Coliform count. The data pertaining to microbial examination and sensory evaluation is shown in Table 13 and Table 14.

Table 12.*Sensory score and microbial quality of orange cordial stored at ambient temperature*

Sr.No.	Days	Sensory score (overall acceptability)	TPC (cfu/ml)	Yeast and Mould count	Coliform count
1	0	8.65	ND	ND	ND
2	15	8.65	ND	ND	ND
3	30	8.60	ND	ND	ND
4	45	8.60	2 x 102	ND	ND
5	60	8.60	3 x 102	1 x 102	ND

Table 13.*Sensory score and microbial quality of orange cordial stored at refrigerated temperature*

Sr.No.	Days	Sensory score (overall acceptibility)	TPC (cfu/ml)	Yeast and Mould count	Coliform count
1	0	8.80	ND	ND	ND
2	15	8.80	ND	ND	ND
3	30	8.75	ND	ND	ND
4	45	8.75	ND	ND	ND
5	60	8.75	1 x 102	ND	ND

Microbiological examination of the sweet orange cordial cordial revealed that coliforms and were found to be absent in the cordial throughout the period of investigation. The yeast and mould appeared after 45 days and TPC appeared after 30 days of storage in case of cordial stored at ambient temperature as shown in Table 12. It is worth mentioning that till 30days there was no colony observed under any microbiological examination in sweet orange cordial kept at refrigerated temperature (Table 13). Thus showing the product was prepared and stored under hygienic conditions.

Sensory evaluation Organoleptic evaluation carried out on every 15th day of storage showed that the sweet orange cordial thus prepared was quite acceptable till the end of the storage period. No observable differences in organoleptic properties were found among the sweet orange cordial at two different temperatures.

4. Conclusions

It can concluded from the above summarized results that the juice extracted can be utilized for various value added products viz RTS, squash and cordial. Their storage quality in physicochemical and microbial can commercialize.

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STUDIES ON PREPARATION OF LOW CALORIE CAKE USING PEARL MILLET (BAJRA) MALTODEXTRIN

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ABSTRACT

An experiment was conducted to develop low calorie food stuff like cake using carbohydrate-based fat replacers. Attempts have made to replace fat in cake by incorporation of pearl millet maltodextrin prepared by acid hydrolysis of pearl millet starch at the rate of 20, 30 & 40 percent. It is recommended to adopt maltodextrin as fat replacer in cake formulation upto the extent of 30 percent as it gives best results on the basis of organoleptic evaluation as compare to experimental control.

Keywords: *Bajra, low calorie cake, maltodextrin ,sensory evaluation*

1. Introduction

Food is a subject of vital interest to everyone in the world.

According to recent survey a majority of consumers showed concerned regarding restrictions in limiting amount of high calorie and cholesterol in daily diet as higher intake of fat is linked with development of cardiovascular disease by American cancer society (ACS-1984).

In search to offer consumers food which are lower in fat content, food technologists have developed several low calorie fat replacement and fat substitutes which functions like oil but less in calorie content.

Fat replacers are mostly carbohydrate or protein based e.g. Polydextrose, maltodextrin, starch derivatives, gums & mucilage and dietary fibres.

They are used to replace fat in foods as they exhibit versatile sensory and textural properties e.g the cakes made with Potato Maltodextrin & emulsifier at either level of replacement were comparable to the control 10 per cent shortening cake by Soberzynska (1991).

In the present investigations efforts have been made to prepare maltodextrin from pearl millet starch and its incorporation in cake in order to prepare low calorie cake.

The usefulness of fat replacers was evaluated by sensory / organoleptic evaluation of low calorie cake by semitrained panelist using hedonic scale.

2. Materials and methods

Bajra (Pennisetum American var. ICTP-8203) grains were procured from NARP, (National Agriculture Research Project, M.A.U., Aurangabad) and these grains were utilized for preparation of Starch.

Quality raw material required for preparation of Cake like Maida, fat, sugar, baking powder, eggs, vanilla essence etc. were procured from local market Parbhani.

Preparation of Maltodextrin (MD) prepared from Pearl Millet (Bajra) Starch (Figure 1).

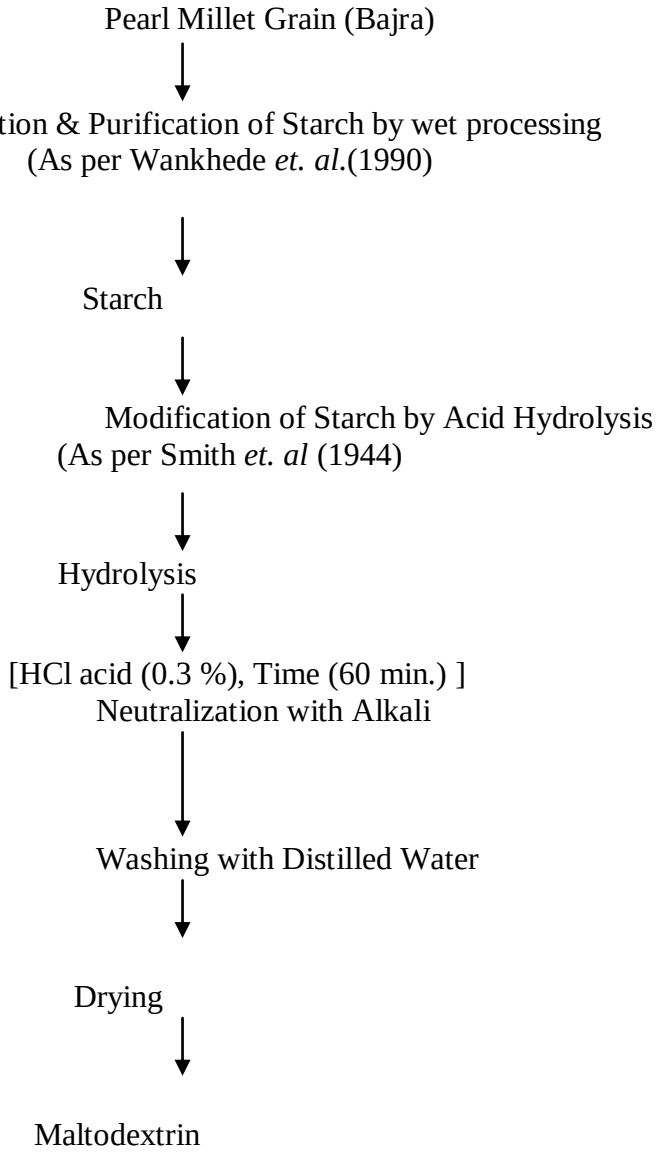

Figure 1: Acid Hydrolysis of Pearl Millet Starch to obtain Maltodextrin.

Fortification of Maltodextrin in Cake

Low Calorie Cake was (Fig. 2) prepared using receipe given by Susan Waring (1998).

Maida	-	120 gm
Sugar	-	120 gm
Fat	-	90 gm
Egg	-	2
Baking Powder	-	2 gm
Vanilla Essence	-	3 ml
Water	-	As required

All the dry ingredients i.e. flour, baking powder and maltodextrin (at the replacement level of 20, 30 & 40 per cent) mixed together and sieved cream fat and sugar are mixed till forming light color paste to this beaten egg is added, dry ingredients are added and mixed uniformly with milk or water to form proper dough.

It is baked at 190 ^{o}C for 15-25 min.

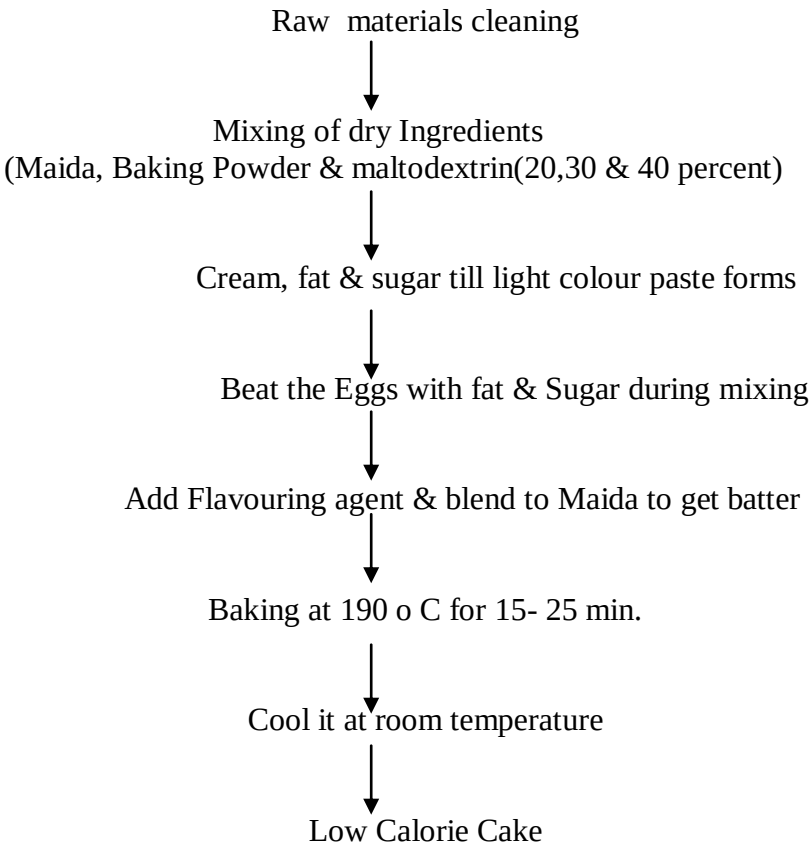

Figure 2. Preparation of Low Calorie Cake

Sensory evaluation of cake

Evaluation of organoleptic attributes of the cakes for color, Flavor, texture, appearance, mouth feel and overall acceptability was done by a semitrained panel of judges. The panel of judges scored on a 9 point scale or Hedonic scale.

Table 1. *Sensory Evaluation of Low Calorie Cake*

Sample Code	Colour	Flavour	Appearance	Mouthfeel	Overall Acceptability
EC	8.1	7.4	7.9	7.7	7.6
A	6.8	6.9	6.1	6.6	6.5
B	8.9	8.8	9.0	8.8	8.7
C	7.4	7.4	7.2	7.0	7.1

Samples EC = Experimental control (Full Fat)
A = 20 per cent Maltodextrin as a fat replacer
B = 30 per cent Maltodextrin as a fat replacer
C = 40 per cent Maltodextrin as a fat replacer

3. Results and discussions

Preparation of Starch & Maltodextrin

In this investigation, the Starch was prepared by using Pearl Millet (Bajra) by wet milling process. The 24 hours soaking time, 750 ppm SO2 at 50 oC temperature resulted into highest yield of Starch (63.20 per cent).

Starch prepared from Pearl Millet (Bajra) was utilized for the preparation of maltodextrin using Hydrochloric acid Hydrolysis. It was observed that acid hydrolysis at 0.3 per cent for 60 min. resulted into highest yield Maltodextrin (95.40 per cent).

Preparation of Low Calorie Cake

The prime objective of the present study was to assess use of Maltodextrin (Acid hydrolyzed as a fat replacer, in low calorie foods like cake. In this investigation Maltodextrin was incorporated during the preparation of cake at the rate of 20, 30 & 40 per cent.

Organoleptic Evaluation of Low Calorie Cake:

4. Conclusions

The average sensory score of each attributes of low calorie cake prepared with incorporation of Maltodextrin at the rate of 20, 30 & 40 per cent & experimental control (without maltodextrin i.e full fat) was recorded in table 1.

The results of sensory evaluation of cake by semi trained panelist showed that the cake prepared using 30 per cent maltodextrin has secured the highest score for each sensory attributes as compared to the experimental control. The actual fat replacement in the finished product was 26.40 per cent. have used maltodextrin and other modified starches as fat replacer to obtain low calorie food stuffs successfully.

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PYSICO-CHEMICAL AND SENSORY CHARACTERISTICS OF WHOLE MILK POWDER STORED AT DIFFERENT TEMPERATURES

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ABSTRACT

The main objective of this research work was to find out the effect of storage conditions on physico-chemical quality of whole milk powder. Whole milk powders of five dairy industries A, B, C, D and E were collected and stored at 40 ^{0}C in polyethylene bags for 120 days. All these treatments were compared with a control (15 ^{0}C) storage temperature. At 15 ^{0}C non significant changes were observed in compositional attributes, pH and acidity. At 40 ^{0}C significant changes were observed in pH, acidity, moisture, free fatty acids, peroxide value, solubility index and hydroxy methyl furfural were recorded, while non significant changes were observed in fat, protein, ash and lactose content.

Keywords: *Whole milk powder, Storage temperature, Hydroxy methyl furfural, free fatty acids, peroxide value.*

1. Introduction

Milk is a perishable food in nature and its shelf life is limited from few days to few hours depending upon the temperature and storage conditions (Kumar and Seth, 2008). Milk powder manufacturing involves the removal of water under hygiene conditions while retaining all the desirable natural properties of the milk - color, flavor, solubility and nutritional value. Whole milk contains 87% water and skim milk contains about 91% water. During milk powder manufacture this water is removed by boiling the milk under reduced pressure at low temperature in a process known as evaporation. The resulting concentrated milk is then sprayed in a fine mist into hot air to remove further moisture and so give a powder. Approximately 13 kg of whole milk powder can be manufactured from 100-kg milk. Milk production in Pakistan in flush season is much more than the requirement (Spreer, 2005). Dairy is one of the expanding industrial sectors in Pakistan; about 17 milk processing units are engaged in the production of various products. Although Pakistan is the 5th largest milk producing country in the world, but there is ample production of milk in winter season and often shortage in summer due to animal reproductive phase (Khan 2005). 16% of the total milk produced in Pakistan is wasted due to the lack of processing and cold storage facilities (Garcia et al. 2007). Surplus milk is available in winter and this is the normal practice of dairy industry in Pakistan that surplus milk is converted in to different types of milk powders (Garcia et al 2007). Most of the imported milk powder is substandard and at the same time milk powder which is produced in our country is also not of good quality. In Pakistan, the duration of load shedding has prolonged and the ambient temperature is also high. So, the fresh milk can not be stored with out refrigeration and at the same time storage of whole milk powder at a suitable environmental condition is necessary. For the reason this project was designed to study the effect of adverse storage conditions on quality of whole milk powder on the basis of certain physico-chemical parameters.

2. Materials and methods

Sample Collection and Storage: The samples of whole milk powder of five dairy industries (T1, T2, T3, T4 and T5) were collected from the Lahore market and stored at 40ºC in polythene bags for 90 days. All these treatments were compared with a control which was stored at 15ºC for the same length of period. All the samples were coded with three digit random number and all the treatments were performed triplicate.

Analysis: Electronic digital pH meter of Wellium model- Inolab pH 720, WTW 82362 was used for pH determination, Fat, protein, lactose, moisture, ash, acidity, hydroxy methyl frurural and solubility index were determined for 90 days at the interval of 30 days by following the respective methods of AOAC (2000). Free fatty acids and peroxide value was determined by using the methods of AOCS (1990)

Statistical analysis: The data was obtained by applying completely randomized design (CRD) and the outcome of the analyses was analyzed through analysis of variance technique Steel et al., (1997). The separation of means or significant difference comparisons was made using Tukey's HSD test.

3. Results and discussion

FAT: The results of fat content of whole milk powder samples stored at 40 ^{0}C are given in table 2. On zero day analysis the highest value of fat (25.75%) in was recorded in E and lowest (25.65%) in C. Variation in fat content in different treatments were statistically non significant (P>0.05). Fat content of all the treatments stored at 40 ^{0}C decreased during storage of 90 days. Maximum decrease in fat was observed at 40^{0}C in T5, fat content decreased from 25.75 (%) to 25.73 (%). And minimum decrease in fat was noted in T3, in which fat decreased from 25.680 to 25.340. The decline in fat content may be due to the hydrolysis and autoxidation of fat, present in higher proportions in whole milk powder. Effect of storage period and storage temperature was statistically non significant. Similar results were reported by (Sadiq, 1999) who reported that fat content decreased during the six month storage of whole milk powder.

Protein: On zero day analysis highest value of protein was exhibited by C (26.29). Protein content non significantly (P>0.05) decreased throughout the storage period at 15 and 40^{0}C but the effect of high temperature was more intense, (0.060%) protein decreased in T1 and T5, while T2 and T3 were less affected. The decline in protein content may probably be due the thermal break down of milk proteins at high storage temperature and absorption of moisture from atmosphere. (Macedo et al. 1997) while studying the storage of milk powder reported that protein decreased during the six months storage of milk powder.

Lactose: On zero day analysis, T4 exhibited the highest value of lactose (33.88%) while T5, showed the lowest value (33.62%). The variation in lactose content within the treatments was statistically non significant (P>0.05). Lactose content decreased in all the samples during the prolonged storage at 15 and 40^{0}C but effects were more deleterious at high temperature (Table 2). The maximum decline was noted in T5 (0.29%) with mean value of (33.33) and minimum decrease in lactose was in T3 (0.20%). Effect of storage period and storage temperature on treatments was statistically non-significant. The decline in lactose content may be due to the growth of mesophillic bacteria which convert lactose into lactic acid. The results of this study in are in line with the findings of

(Goodnaught and kleyn, 1976) who reported that decrease in lactose content during storage of whole milk powder is due to the production of lactic acid.

Table 1: *Effect of storage on Physico chemical characteristics of whole milk powder at 15 ^{0}C*

Storage Period (Days) T1	Fat %	Protein %	Lactose %	Ash %	Moisture %	pH	Acidity %	Free Fatty Acids%	Per oxide value Meq/Kg	Solubility Index %	Hydroxy methyl Furfural %
0	25.680	26.220	33.680	5.760	3.650	6.610	0.171	0.088	0.153	0.943	0.521
30	25.680	26.210	33.680	5.760	3.660	6.610	0.169	0.088	0.155	0.950	0.521
60	25.660	26.210	33.660	5.750	3.670	6.607	0.170	0.090	0.156	0.960	0.521
90	25.630	26.210	33.650	5.750	3.680	6.600	0.172	0.090	0.158	0.965	0.521
120	25.580	26.200	33.650	5.740	3.700	6.597	0.178	0.092	0.169	0.960	0.522
Mean	25.646	26.210	33.664	5.752	3.672**c**	6.605	0.171**d**	0.089**de**	0.158**h**	0.956**c**	0.521**c**
S.D.	± 0.566	±0.517	±0.768	±0.194	±0.059	±0.199	±0.005	±0.003	±0.171	±0.109	±0.024
Storage Period (Days) T2											
0	25.700	26.250	33.810	5.740	3.740	6.710	0.175	0.081	0.200	0.950	0.508
30	25.690	26.250	33.810	5.740	3.740	6.710	0.176	0.082	0.205	0.960	0.508
60	25.680	26.240	33.800	5.730	3.750	6.709	0.178	0.082	0.207	0.960	0.508
90	25.660	26.230	33.780	5.720	3.770	6.703	0.181	0.083	0.210	0.970	0.508
120	25.620	26.230	33.760	5.720	3.790	6.703	0.183	0.083	0.212	0.970	0.508
Mean	25.620	26.230	33.792	5.730	3.758**b**	6.707	0.179**d**	0.082**f**	0.206**fg**	0.962**c**	0.508**d**
S.D.	±0.521	±0.662	±0.109	±0.202	±0.104	±0.147	±0.005	±0.003	±0.173	±0.095	±0.011
Storage Period (Days) T3											
0	25.650	26.290	33.760	5.770	3.680	6.757	0.169	0.052	0.105	0.800	0.496
30	25.660	26.300	33.760	5.780	3.690	6.760	0.170	0.052	0.106	0.800	0.496
60	25.640	26.290	33.740	5.770	3.700	6.757	0.173	0.053	0.108	0.817	0.495
90	25.610	26.280	33.730	5.760	3.720	6.6750	0.172	0.054	0.112	0.817	0.495
120	25.580	26.280	33.710	5.760	3.730	6.750	0.173	0.054	0.112	0.820	0.496
Mean	25.628	26.288	33.740	5.768	3.704**c**	6.616**c**	0.173**d**	0.053**h**	0.108**i**	0.811**d**	0.496**d**
S.D.	±0.903	±0.792	±0.601	±0.186	±0.122	±0.177	±0.006	±0.002	±0.178	±0.040	±0.015
Storage Period (Days) T4											
0	25.720	26.180	33.880	5.790	3.750	6.7550	0.165	0.103	0.176	0.820	0.560
30	25.710	26.170	33.880	5.780	3.760	6.755	0.166	0.104	0.178	0.8430	0.560
60	25.700	26.170	33.870	5.780	3.770	6.752	0.176	0.104	0.179	0.8430	0.560
90	25.650	26.160	33.850	5.770	3.790	6.747	0.177	0.105	0.181	0.853	0.561
120	25.610	26.150	33.820	5.770	3.800	6.742	0.180	0.106	0.184	0.860	0.561
Mean	25.678	26.166	33.860	5.778	3.774**ab**	6.750	0.172**d**	0.104**c**	0.180**fgh**	0.844**d**	0.560**b**
S.D.	±0.634	±0.566	±0.821	±0.186	±0.107	±0.215	±0.006	±0.004	±0.183	±0.050	±0.018
Storage Period (Days) T5											
0	25.750	26.200	33.620	5.720	3.790	6.734	0.178	0.084	0.250	0.870	0.615
30	25.740	26.200	33.610	5.720	3.800	6.733	0.178	0.084	0.251	0.880	0.615
60	25.740	26.190	33.600	5.710	3.820	6.730	0.180	0.085	0.254	0.890	0.615
90	25.700	26.190	33.580	5.710	3.830	6.725	0.182	0.087	0.255	0.897	0.616
120	25.650	26.180	33.560	5.700	3.840	6.720	0.184	0.088	0.257	0.897	0.617
Mean	25.716	26.192	33.594	5.712	3.816**a**	6.729	0.180**d**	0.086**ef**	0.253**de**	0.887**d**	0.616**a**
S.D.	±0.615	±0.418	±1.034	±0.177	±0.104	±0.225	±0.006	±0.004	±0.190	±0.045	±0.030

Means carrying same expression in column are statistically non-significant

Ash: Ash content ranged from 5.20 to 5.790 between different treatments on zero day analysis and was statistically at par (P>0.05). Ash content were not influenced by storage temperature and after 120 days of storage at 15 and 40^{0}C there was as slight decrease in ash content of all the samples of whole milk powder. Maximum decrease (0.040%) was in A, D and E, while a decline of (0.02 %) was seen in B and C (Table 1). The decrease in ash content may be due to the absorption of moisture by whole milk powder during prolonged storage. Sadiq (1999) reported ash content of whole milk powder was (5.621).

Moisture: As can be seen from (Table 1) that variation in moisture content within different samples of whole milk powder was statistically significant (P<0.05). Moisture content ranged from 3.650 to 3.790 between different samples of whole milk powder. After 120 days moisture content increased in all the samples stored at 15 and 40^{0}C, the increase in moisture content was almost uniform in all the samples. Effect of storage period and temperature on moisture was found statistically non-significant. The increase in moisture content may be due to the permeability of polyethylene to moisture and oxygen. The results of this study are in

close agreement with the findings of (Semenuic et al. 2008) they studied the the pysico chemical changes in whole milk powder during different storage conditions (ambient and 15^0C) for 18 months and reached conclusion that titratble acidity and moisture increased with the storage time in whole milk powder.

Table 2: *Effect of storage on Physico chemical characteristics of whole milk powder at 40 ^{0}C*

Storage Period (Days) T1	Fat %	Protein %	Lactose %	Ash %	Moisture %	pH	Acidity %	Free Fatty Acids%	Per oxide value%	Solubility Index %	Hydroxy methyl Furfural%
0	25.680	26.220	33.680	5.760	3.650	6.610	0.171	0.088	0.153	0.943	0.521
30	25.570	26.210	33.640	5.750	3.670	6.603	0.175	0.093	0.306	0.977	0.528
60	25.490	26.200	33.600	5.740	3.670	6.597	0.183	0.103	0.557	1.150	0.535
90	25.380	26.190	33.550	5.730	3.680	6.578	0.190	0.114	0.721	1.154	0.546
120	25.340	26.160	33.470	5.720	3.700	6.540	0.199	0.131	0.863	2.080	0.553
Mean	25.472	26.196	33.588	5.744	3.674**c**	6.585	0.183**c**	0.05**db**	0.520**h**	1.312**b**	0.536**c**
S.D.	± 0.628	±0.757	±0.855	±0.212	±0.108	±0.185	±0.012	±0.016	±0.659c	±0.446	±0.017
Storage Period (Days) T2											
0	25.700	26.250	33.810	5.740	3.740	6.710	0.175	0.081	0.200	0.950	0.508
30	25.650	26.240	33.770	5.730	3.740	6.690	0.179	0.096	0.435	0.83	0.509
60	25.560	26.220	33.720	5.730	3.760	6.658	0.184	0.107	0.612	1.180	0.515
90	25.460	26.220	33.680	5.720	3.770	6.650	0.200	0.125	0.895	1.440	0.523
120	25.320	26.210	33.580	5.720	3.780	6.639	0.206	0.114	1.050	2.250	0.534
Mean	25.538	26.228	33.718	5.734	3.758**b**	6.660	0.189**b**	0.121**a**	0.639**b**	1.353**b**	0.517**c**
S.D.	±1.100	±0.600	±0.993	±0.191	±0.107	±0.161	±0.013	±0.022	±0.173	±0.500	±0.017
Storage Period (Days) T3											
0	25.650	26.290	33.760	5.770	3.680	6.757	0.169	0.052	0.105	0.800	0.496
30	25.640	26.280	33.720	5.760	3.690	6.751	0.174	0.061	0.236	0.810	0.502
60	25.490	26.260	33.650	5.760	3.700	6.748	0.178	0.069	0.395	0.933	0.508
90	25.470	26.270	33.620	5.760	3.720	6.745	0.183	0.079	0.632	1.085	0.518
120	25.221	26.250	33.560	5.750	3.730	6.740	0.196	0.085	0.783	1.547	0.524
Mean	25.494	26.270	33.662	5.714	3.704**c**	6.748	0.180**b**	0.069**f**	0.430**c**	1.028**c**	0.510**c**
S.D.	±1.072	±0.707	±0.940	±0.194	±0.113	±0.176	±0.017	±0.013	±0.706	±0.332	±0.024
Storage Period (Days) T4											
0	25.720	26.180	33.880	5.790	3.750	6.655	0.165	0.103	0.176	0.820	0.560
30	25.670	26.180	33.820	5.790	3.760	6.655	0.170	0.113	0.156	0.990	0.566
60	25.600	26.160	33.750	5.790	3.770	6.621	0.175	0.125	0.766	1.140	0.572
90	25.500	26.150	33.700	5.760	3.790	6.580	0.185	0.137	0.936	1.470	0.582
120	25.350	26.130	33.610	5.750	3.800	6.553	0.196	0.161	1.115	2.117	0.588
Mean	25.568	26.160	33.752	5.774	3.774**ab**	6.513	0.178**cd**	0.128**a**	0.650**b**	1.319**b**	0.574**b**
S.D.	±0.783	±0.568	±1.287	±0.268	±0.091	±0.245	±0.012	±0.021	±0.595	±0.503	±0.018
Storage Period (Days) T5											
0	25.750	26.200	33.620	5.720	3.790	6.734	0.178	0.084	0.250	0.870	0.615
30	25.700	26.190	33.560	5.710	3.800	6.728	0.180	0.092	0.519	0.997	0.623
60	25.620	26.170	33.510	5.710	3.820	6.719	0.191	0.105	0.852	1.150	0.626
90	25.510	26.160	33.430	5.700	3.830	6.709	0.190	0.119	0.993	1.350	0.636
120	25.380	26.140	33.330	5.680	3.840	6.695	0.213	0.142	1.198	2.170	0.646
Mean	25.592	26.172	33.642	5.704	3.816**a**	6.717	0.190**b**	0.108**b**	0.760**a**	1.307**b**	0.629**a**
S.D.	±0.887	±0.540	±1.103	±0.161	±0.099	±0.235	±0.015	±0.027	±0.511	±0.485	±0.022

Means carrying same letter in column are statistically non-significant

Acidity: The results regarding acidity of whole milk powder samples stored at 15, and 40 ^{0}C temperatures for 120 days are given in (Table2). Acidity ranged from 0.165 to 0.178 between different samples. On zero day analysis highest acidity 0.178 was recorded in T5. The least value of acidity 0.165 was noted in T4. After 120 days acidity of all samples stored at 15 and 40 ^{0}C increased. This could be the reason of multiplication of bacteria which converts the lactose into lactic acid. Acidity is associated with storage of whole milk powder; higher levels indicated poor keeping quality. Effect of storage period and temperatures had highly significant effect on acidity of whole milk powder samples. Similar findings were reported by Semeniuc et al. (2008). pH: On zero day analysis pH ranged from 6.755 to 6.610 between different samples of whole milk powder (Table1). pH decreased throughout the storage period of 120 days in all samples and at different storage temperatures. Effect of temperature on pH was found significant ($P<0.05$). The decrease in pH of whole milk powder samples may be due to the growth of psychrophillic and thermophillic bacteria. Similar results were

reported by (Akhter et al. 2003) they reported that pH of whole milk powder samples stored at ambient temperature for 30, 60 and 90 days containing 4.49 percent moisture decreased during 90 days of storage.

Free Fatty Acids: Free fatty acids ranged from 0.054% to 0.102% between different treatments. Free fatty acids significantly increased throughout the storage period of 120 days at different storage temperatures 15 and 40 ^{0}C. On zero day analysis highest value of free fatty acids (0.103%) was recorded in the T5, the least value (0.052%) was noted in T3. At 15 ^{0}C storage temperature for 120 days maximum increase in free fatty acids was reordered in treatment T1 and T5 (0.004%) and minimum increase in samples T2 and T3 (0.002%) . At 40 ^{0}C for 120 days, maximum increase in free fatty acids (0.058) in T4 and T5 and minimum increase (0.033%) was observed in T3. The free fatty acids increased throughout the storage period in all treatments at all storage temperatures. The increase was probably due to hydrolysis of fat during storage. Variation in treatments was highly significant and the effects of treatment, storage time and storage temperatures on free fatty acids were found also highly significant. The results of this study are in close association with finding of (Paez et al. 2007) who reported that by storing whole milk powder at 21 and 40 ^{0}C free fatty acids increased by 0.012 and 0.031 respectively. Short chain free fatty acids are more volatile and responsible for the development of rancid flavor in whole milk powder. Free fatty acids are directly related with shelf life of fats. Higher values indicated higher levels of oxidative break down and reduced shelf life.

Peroxide Value: Peroxide value ranged from 0.10 to 0.25 (Meq/Kg) between different treatments of whole milk powder. On zero day analysis highest values of peroxide (0.25) was recorded in the sample T5 and the least value of peroxide (0.105) was noted in T3. The increase in per oxide value may be due to the oxidation of milk fat and reaction is catalyzed by high storage temperature (Table-2). At 15 ^{0}C storage temperature for 120 days maximum increase in peroxide value was reordered in T1 (0.017%) and minimum increase in treatment C and E (0.007) was noted. At 40 ^{0}C storage temperature for 120 days, maximum increase in peroxide value (1.148) in E and minimum increase (0.627) in C was observed. The results of this study are in close association with the findings of Fayyaz (2000) who reported that by storing whole milk powder at 21 and 40 ^{0}C per oxide value increased from 0.15 and 1.67 during storage of 3 months. Peroxide value indicates the degree of oxidation in the fat. It is directly associated with keeping quality of foods higher levels indicated short shelf lives.

Solubility Index: Results of solubility index are given in Table 2. On zero day analysis the maximum value of solubility index (0.95%) was recorded in T2 and the lowest value (0.80%) was noted in T3. At 15 ^{0}C for 120 days maximum increase in solubility index was reordered in treatment T4 (0.02) and minimum decrease in treatment T1 and T3 (0.02%) was noted. At 40 ^{0}C for 120 days, maximum increase in insolubility index (1.33%) in T4 and minimum increase (0.73%) in treatment T3 was observed. Solubility index increased through out the storage period. The increase in solubility index may probably be due to the thermal break down of milk powder proteins at high storage temperature. Similar results were reported by (Semeniuc et al. (2008) they reported that during the storage of whole milk powder for 18 months solubility index increased. Hydroxy Methyl Furfural: On zero day analysis highest values of hydroxy methyl furfural (0.61%) was recorded in the sample T5,

the minimum value of hydroxy methyl furfural (0.49%) was noted in T3 (Table-2) At 40 ^{0}C storage temperature for 120 days, maximum increase in hydroxy methyl furfural (0.016) in T1 and minimum increase (0.013) in treatment T3 and T4 was observed. Hydroxy methyl furfural increased during the storage of 120 days at 15 ^{0}C and 40 ^{0}C but the higher levels were found at 40 ^{0}C. The results of this study are in close agreement with the findings of (Soledad et al. 1998) who reported an increase in hydroxy methyl furfural (HMF) during the storage of infant milk powder for 9 months at 20, 30 and 37 ^{0}C while no change in available lysine occurred during storage at any temperature. Hydroxy methyl furfural is a product of heat induced reaction between amino acids and lactose, the reaction is accelerated at higher temperatures, so hydroxyl methyl furfural indicates the process conditions in which milk products are manufactured. Higher levels indicated that milk products are processed at high temperature for a longer period of time.

4. Conclusions

The main objective was to find out the effect of adverse storage temperatureon the physic chemical characteristics of whole milk powder. As it is evident from the analysis (Table 2) that quality was remarkably decreased at high temperature. Peroxide value, free fatty acids, moisture, pH, acidity, hydroxyl methyl furfural and solubility index were significantly affected and the product became unacceptable. Quality of whole milk powder stored at 15 ^{0}C was satisfactory after storage of 90 days, It may be recommended that whole milk powder produced in Pakistan can be kept well for three months at 15^{0}C.

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Short communication

EFFECT OF DIFFERENT FAT LEVELS ON SOME QUALITY PARAMETERS OF GOUDA CHEESE FROM SHEEP MILK

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ABSTRACT

A study was carried out to find out the effect of different fat levels on physicochemical and sensory characteristics of Gouda cheese from sheep milk. This study was carried out in PTC- Plus, Netherlands. Gouda cheese was prepared from sheep milk using three different fat levels i.e. T1 (6% fat) T2 (5% fat) and T3 (4%fat) other ingredients were same in all treatments. Gouda cheese was prepared by using standard Dutch recipe and stored for 90 days at 12 -15 0C and storage study was conducted at the interval of 30 days. Fat and protein content increased from 41.22 to 43.05 and 22.25 to 24.0% respectively, moisture content decrease through out the storage period of 90days. Sensory evaluation scores indicated T3 (4% fat) was rated best from taste, flavor and overall acceptability view points. It may be concluded that good quality Gouda cheese can be made from sheep milk using 4% fat in cheese milk.

Keywords: *Gouda Cheese, Sheep Milk, Fat, overall acceptability*

1. Introduction

Pakistan has a large livestock population having 30.5 million sheep. More than 50% of sheep are reared in the western dry mountains, western dry plateau and northern dry mountains (otto et al. 2002). Cheese is produced throughout the world in wide-ranging flavors, textures, and forms. Cheese consists of proteins and fat from milk, usually the milk of cows, buffalo, goats, or sheep. It is produced by coagulation of the milk protein casein. Typically, the milk is acidified and addition of the enzyme rennet causes coagulation. The solids are separated and pressed into final form. It is believed that goat and sheep milk were the first milks used to prepare cheese. Cheese is high in calcium and phosphorous content and very low in lactose content. Cheese is rich in vitamin-B, which is very good for children, women (particularly pregnant and lactating) and elderly people, for formation and strengthening of bones and cartilages. Cheese contains Conjugated Linoleic Acid and Sphingolipids which help prevent cancer (Ulfman, 1996) Gouda is a dutch type cheese and win second best cheese prize in world cheese competition held in 2002 (Hellner, 2002). The present project was designed to develop Gouda cheese from sheep milk to convert sheep milk of Pakistan into value added dairy product to improve sheep production and economic conditions of sheep herd owners.

2. Materials and methods

Raw sheep milk was purchased from a farm located in Gystjerk, The Netherlands. Sheep milk was analyzed for fat, protein, lactose, ash contents, SNF and total solids by using Lactoscope, Delta Instruments, Holland. Starter Culture CSK G300, Liquid Rennet, Calcium Chloride and Salt Peter was purchased from CSK, Foods Friesland Holland. Milk was divided into three batches and standardized to 4, 5 and 6% fat content. Mother culture 1% was prepared by using CSK G300. Milk was pasteurized in batch pasteurizer at 65 ^{0}C for 30 minutes and immediately cooled to 30 ^{0}C. 5 mL starter culture was added in

each batch of standardized sheep milk and pre acidification was carried out for 30 minutes. 2mL calcium Chloride (35%), 1mL salt peter and 1mL rennet was added and mixed for three minutes and then allowed to coagulate for 30 minutes. Coagulum was cut into 1 centimeter cubes, 40% whey was drained and curd was continuously stirred to prevent the formation of big lumps. 1.2 liter hot water (41 ^{0}C) was added into the curd to increase the temperature of curd. Temperature of curd was maintained at 36 ^{0}C for 30 minutes and then 20% of whey was removed by keeping strainer on the valve of cheese vat. Curd was transferred into 0.5kg molds and pressed at 0.5kg bar for 30 minute and then pressure was increased to 2 bar and removed from press after 1.5 hours. Cheese was left at room temperature for 14 hours and then transfer into brine bath (NaCl 20 %) and taken out from brine after 8 hours and left for drying on wooden shelf for 24 hours and in next two days plastic coating was carried out on both sides and cheese was ripened for 90days. Cheese samples were analyzed for fat, protein, moisture and pH by following the methods (A.O.A.C., 1990; Kirk and Sawyer, 1991). Organoleptic evaluation of cheese was carried out by using 9 point Hedonic scale according the method of Larmond (1977). Data was statistically analyzed by using completely randomized design and comparison of mean difference was carried out by using Duncan Multiple Range Test (DMR Test) as described by steel et al. (1997)

Table 1- *Effect of Different Levels of Fat on Composition of Sheep Milk Gouda Cheese*

Treatments	**Fat(%)**	**Moisture(%)**	**Protein(%)**	**Fat in Dry Matter(%)**
T_1 (6% Fat)	48.26±2.15^a	30.12±1.19^c	19.65±0.42^c	69.06±4.33^a
T_2 (5% Fat)	42.11±1.65^b	32.51±1.22^b	23.56±0.52^b	62.39±3.89^b
T_3 (4% Fat)	34.79±1.26^c	40.02±1.58^a	25.19±0.81^a	58.00±2.89^c

Table 2- *Effect of Storage on Chemical Composition of Sheep Milk Gouda Cheese*

Storage (Days)	**Fat(%)**	**Moisture(%)**	**Protein(%)**	**Fat in Dry Matter(%)**
0	41.22±1.63^b	34.01±1.23^a	22.25±0.65^c	62.46±3.91^b
30	40.75±1.61^c	33.22±1.18^b	22.74±0.69^c	61.02±3.77^c
60	41.79±1.75^b	33.05±1.10^b	23.56±0.73^b	62.41±3.98^b
90	43.05±1.84^a	32.45±1.04^c	24.03±0.79^a	64.47±4.11^a

Table 3- *Effect of Different Levels of Fat on Sensory Characteristics of Sheep Milk Gouda Cheese*

Treatments	**Taste**	**Flavor**	**Texture**	**Overall Acceptability**
T_1 (6% Fat)	7.2±0.68^c	7.5±0.74^b	7.1±0.51^a	7.5±0.44^b
T_2 (5% Fat)	7.9±0.89^b	7.7±0.65^a	7.0±0.39^a	7.6±0.49^b
T_3 (4% Fat)	8.3±0.95^a	7.5±0.49^b	7.2±0.60^a	7.8±0.85^a

Table 4- *Effect of Storage on Sensory Characteristics of Sheep Milk Gouda Cheese*

Storage (Days)	Taste	Flavor	Texture	Overall Acceptability
0	7.9±0.89[c]	7.5±0.71[c]	7.0±0.41[c]	7.5±0.61[b]
30	8.2±0.91[b]	8.0±0.85[b]	7.4±0.66[b]	7.6±0.24[b]
60	8.3±0.90[a]	8.1±0.79[b]	7.5±0.58[b]	7.8±0.19[b]
90	8.4±0.82[a]	8.3±0.65[a]	7.8±0.49[a]	8.0±0.55[a]

3. Results and discussion

Physico-Chemical Analysis: The results of physicochemical parameters of different types cheese is given in Table 1 which indicated that fat content of cheese progressively increased as the fat content of cheese milk was increased, protein content and moisture content increased by decreasing fat content of cheese milk. Fat content of cheese in all the treatments were more than prescribed standards of gouda cheese (minimum 48% fat on dry matter basis). Analysis of variance indicated that all the treatment were significantly different from each other and effect of storage on treatments was also significant. At the end of storage period (90 days) fat and protein content increased, this may be due to evaporation of water from cheese. Similar results were reported by Jabbar et al.(2003) and Tarannum (1986) and Licitra et al. (2000).

Sensory Evaluation: Sensory evaluation of different treatment of gouda cheese indicated that T3 is highly rated rated for taste and overall acceptability followed by T2 which obtained maximum score for flavor. Score for sensory evaluation increased through out the storage period of 90 days. This may be due to the development of better aroma and flavor of cheese during ripening. During storage of 90 days no visible growth of fungus, early and late blowing of cheese was observed. Similar results were reported by jabbar et al.(2003)during the preparation of gouda cheese from buffalo milk.

4. Conclusions

The main objective of this research work was to develop Gouda Cheese from sheep milk at different fat levels. As can be seen from the scores of sensory evaluation that T3 (4% fat) was highly rated for flavor, taste and texture. It is concluded that good quality Gouda Cheese can be made from sheep milk using 4% fat in cheese milk.

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Short communication

ABNORMALLY HIGH URINARY BISPHENOL - A LEVELS IN HUMANS FROM CANNED SOUP INTAKE. WHAT IS IT SUGGESTIVE OF?

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ABSTRACT

Bisphenol-A (BPA) is usually associated with plastics leachate and most of the research has been primarily focused on identifying ways and means to prevent the same . However, there are new indications from recent studies that consumption of canned products especially soups can increase the urinary BPA levels in humans by more than 1000%. The potential health effects of BPA on human body are still under debate in scientific communities at this point. As Benjamin Franklin rightly said " An ounce of prevention is better than a pound of cure", so as a precaution for human health & development, it is highly advisable to regulate the use of Bisphenol-A in metal food containers which is finding its way directly to our homes, onto our dining tables through canned food products like fish, beans, corn, soups, dry milk and others, especially when such products are widely available for consumption by communities of all ages and wellbeing incl. healthy ,sick, pregnant, at-risk and hospital communities at large. It is time that the options for BPA-free canned food packaging be considered.

Keywords: *Bisphenol A, Food preservative, Canned food, Metal cans lining, Health hazards, Food packaging, Exposure, Endocrine Disruptor, Carcinogenic, BPA-free food packaging.*

BPA exposure to humans through consumer products

Bisphenol-A(BPA) [2,2-bis(4 hydroxyphenyl)propane; CAS no. 80-05-7] is produced in the world in very high volumes upto an amount of 6 billon pounds per year, out of which around 1 million pound or more per year is manufactured or imported only in United States (U.S. Environmental Protection Agency 2004).

It is used in the plastic industry, metal food containers industry, and as composites and sealants in dentistry [Center for the Evaluation of Risks to Human Reproduction (CERHR) 2007, European Union 2003,Burridge 2003].

It is used for the manufacture of epoxy resins, for application in metal food cans as a protection against rusting and corrosion.

There are documented studies about potential migration of BPA leaching from epoxy resins coatings to the food in the metal cans(Vandenberg et al 2007).

This is not the only exposure route to humans, others being drinking water, composites and sealants in dental industry, skin exposure and dust inhalation. Every year around 100 tons of BPA is accidentally released into the air during production (Stahlhut 2009,Kang et al 2006,Vandenberg et al 2007).

It is estimated that 9 out of 10 persons in the world are exposed to BPA (Calafat et al 2005,2008) (Vandenberg et al 2007) .

Human Health Effects from BPA Dietary intake exposure

The last decade of studies: A Debate

Studies clearly indicate that higher the BPA Concentration in urine, more adverse are the health effects on humans. These hypotheses were based on animal and laboratory evidence , which clearly report that half life for renal clearance of BPA after oral ingestion is 5.3 hours (Male & female subjects)(Volkel et al 2002). The oral ingestion of BPA results in metabolization to Bisphenol A-glucuronide , associated with adverse effects on liver, cardiovascular disease and obesity(Volkel et al 2002).

US National Health and Nutrition Survey (NHANES) 2003/04 released epidemiological data on a study of urinary BPA concentrations and their health effects on a large-scale population. It clearly showed that higher the urinary BPA concentration, more adverse health problems associated with cardiovascular disease and diabetes(Lang 2008,Melzer 2008,2009,Carwile et al 2011).

Moreover in animal studies, it was demonstrated that high BPA doses result in estrogen like effects on uterine and prostate organ weights. With low doses, below the putative lowest observed adverse effect level, the results indicated decrease in sperm production, increase in prostate gland volume, changes in the mammary gland, changes in altered vaginal morphology and estrous cycles, disruption of sexual differentiation in the brain, and accelerated growth and puberty (Durando et al. 2007; Howdeshell et al. 1999; Kubo et al. 2003; Richter et al. 2007; Rubin et al. 2006; Schonfelder et al. 2002; Timms et al. 2005; Vom Saal et al. 1998; Vandenberg et al 2009). Presently, the subject of low dose effects of BPA is under contest in scientific communities(European Union 2003; Goodman et al. 2006; Gray et al. 2004; National Toxicology Program 2001; Vom Saal et al 2005), however there are recent studies(Welshons et al 2006) that clearly indicate various pathways through which even low dose BPA can stimulate cellular response to bind to the classical nuclear or genomic Estrogen Receptors.

Studies suggesting Otherwise

However, there are research studies that do not agree with the above conclusions. Willhite et al (2008) indicated that there is no for immunologic ,neurologic or carcinogenic risk for humans by oral ingestion of BPA. The study was conducted on urinary samples of 205 human subjects (men & women) and clearly ruled out BPA as endocrine disruptor based on the urinary BPA conjugates.

The Debate

A lot of studies have been carried out since 1999 that used different techniques and experimental methods to measure unconjugated BPA in human serum, which has been identified to be present in the range of 0.2-20 ng/ml , thus ruling out the potential theory of only oral ingestion route (Vandenberg et al 2007).

Thus, the route of human exposure to BPA is strongly contested. Studies by Vandenberg et al (2007), Willhite et al (2008) and Welshons et al (2006) indicate differences in metabolic pharmacokinetics, after oral contact, skin contact and intravenous exposure of humans to BPA. The proposed exposure route of humans to BPA may be by bathing with BPA-Contaminated water, by inhaling BPA-Contaminated air, or by implanted medical devices and tubes. Definitely , there is more than oral ingestions route which is causing BPA exposure. Such studies which suggest that only oral ingestion is possible route for humans failed to take in account other possibilities of contamination (Willhite et al 2008, Vandenberg et al 2007).Not only that, there are confirmed studies indicating the ability of BPA to cross human placental barrier (Vandenberg et al 2006, Schonfelder et al 2002).Experiments indicate that lower level of BPA can

regularly detected in human blood, thus agreeing to the above stated fact.

When unconjugated BPA is repeatedly detected in human fluids and tissues, it clearly tells that BPA is not rapidly metabolized and remover from human body. Thus to determine the pharmokinetics of BPA metabolism in human subjects , a sensitive approach is definitely needed. Experimental studies clearly indicate the presence of unconjugated BPA in most human tissues incl. human placenta (Vandenberg et al 2009).

Some of the recent studies:

Studies in 2008

A cancer research concluded the apoptotic effects of BPA in three different acute myeloid leukemias (Bontempo et al 2008). Another study in 2008 clearly related the correlation of fetal exposure to BPA and increased incidence of Breast Cancer over the last five decades in US and Europe. It clearly concluded the fact that fetal environment is sensitive to BPA exposure and can lead a breast cancer in adulthood(Soto et al 2008). The study , carried out at California Pacific Medical Center Research Institute, San Francisco, CA also explored the possibility of correlation of prior exposure to BPA , maintaining tumor aggressiveness and poor patient outcome.(Dairkee et al 2008).

Studies in 2009

A study in 2009 clearly identified the connection between low dose exposure of BPA and detrimental effects on human placental cells ,leading in vivo to adverse pregnancy outcomes such as preeclampsia, intrauterine growth restriction, prematurity and pregnancy loss.(Binochour & Aris 2009).

Studies in 2010

A cancer research study connected the risk of cancer initiation in human body by direct and/or indirect mechanisms including DNA mutations to BPA exposure (Cavalieri & Rogan 2010).Another significant study clearly indicated the ability of Bisphenol-A to induce neoplastic transformation in human breast epithelial cells(Fernandez & Russo 2010). Study by Zhu et al (2010) suggested the role of BPA in causing Neuroblastoma cell proliferation. Neuroblastoma (NB) is the most common pediatric extracranial cancer . Another study on the effects of BPA exposure to placental and fetal development linked the same to reproductive toxicity(Mørck et al 2010).

Studies in 2011

A recent study on mice concluded the risk of developing mammary cancer in mice through BPA exposure(Weber & Keri 2011). In a study carried out by Lawson et al (2011), there are experimental evidence which point out that exposure to BPA leads to adverse reproductive effects in both males and females.

BPA in metal-canned food products

Bisphenol-A is present in many consumer food products , packaged in metal cans.It is used as an interior coating to prevent rust and corrosion to the metal can packaging(Carwile et al 2011). According to the European Food Safety Authority (EFSA), the current tolerable daily intake value (TDI) of BPA is 0.05 mg/kg body weight.(Hengstler et al 2011). According to a study carried out in Quebec city, Canada , around 154 consumer canned food products were assessed for the presence of BPA. The analysis showed that BPA was present in around 36 % (Cao et al 2010). High concentrations of BPA were found mostly in the composite samples containing canned foods, with the highest BPA level being observed in following products :

Fish : 106 ng/g, Corn : 83.7 ng/g, Soup :22.2–44.4 ng/g, Baked beans:23.5 ng/g, Peas:16.8 ng /g, Dry milk:15.3 ng/g, Meats:10.5 ng/g). What is interesting is that 19 out of 55 samples, account for more than 95% of the total dietary intakes, and most of the 19 samples were either canned or in jars. Also, Intakes of BPA from non-canned foods are low.

Recent study on canned soups

A recent study carried out by Harvard School of Public Health concluded an increase in more than 1,200% in urinary BPA levels compared with the consumption of fresh soup daily for the same period(Carwile et al 2011). The study subjects were made to consume soup directly out of metal cans for five days and thus ruled out any external contamination of BPA with the subjects. Normally urinary BPA levels in typical adults average somewhere around 2 micrograms per liter. However, in this study, the urinary BPA levels displayed a rise above 20 micrograms per liter , a 1221% increase from the study subjects who consumed soup from fresh ingredients. Another study evaluated the contribution of food packaging to human-BPA exposure using dietary intervention on human subjects. It measured urinary BPA and phthalate metabolites before, during, and after dietary intervention of fresh foods. The urinary analysis results showed decreased levels of BPA and DEHP metabolites during the fresh foods intervention in the human subjects(Ruthann et al 2011).

Conclusions

It is evidentally clear from recent studies that use of epoxy coatings in metal cans is responsible for high urinary BPA concentrations in humans, however serious health effect concerns are unclear and debatable. It does not suggest in any way that we can have a can of BPA contaminated food product every morning and evening and expect to lead a healthy life. Quite clearly, the data collected so far in the field of environmental toxicology is good enough to alert us about the future impact of Bisphenol-A on human health & development.

BPA is being used in the packaging of very high demand consumer food products. These products are widely available to healthy, unhealthy, pregnant, at-risk and even hospital communities as a substitute for fresh food ingredients. Based on the experimental evidence and scientific theories available to us, the regulatory agencies should substitute the chemicals like BPA to safer options that are not likely to be harmful to the normal development of humans . The National Toxicology Program report, statement by the FDA's commissioner, and a report from Health Canada , all classified BPA as a human and environmental toxin, which should be used as a platform to regulate BPA exposure. Such abnormally high levels of BPA in human urine clearly suggest the consideration for BPA-free food products. That would be possible only by exploring the possibilities of a BPA- free packaging for a healthy future of consumers.

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