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Zinc and iron levels in pregnancy: A review

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Abstract

Zinc and iron are micronutrients whose requirements increase with pregnancy. Zinc is so crucial to primary immunologic function in pregnancy that its deficiency may be lethal in both human and animals. Iron is needed to be supplement in pregnancy in order to avert anaemia due to pregnancy. During pregnancy a woman needs additional iron to satisfy the demand of the fetus, the placenta and her increasing hemoglobin mass. This review is aimed at x-raying the significance of these mineral elements especially during pregnancy with a view to educating readers on the likely course of infant and maternal death especially in developing countries.

Keywords: pregnancy, iron, zinc, deficiency, level

Introduction

Scientific attention to the role of specific nutrients especially micronutrients in healthy pregnancy functioning has grown steadily in recent years, beginning with numerous fields of studies of pregnancy in developing countries, which gathered sad testimony to the harm done by malnutrition to the body's resistance to disease. The risk of this complication is doubled in pregnancy. The trace metal zinc is so crucial to primary immunologic function in pregnancy that its absence may be lethal in both human and animal systems (McKenzie, 1998). Studies of nutrients have evolved out of earlier studies on pregnant women with protein-calorie malnutrition, both clinical and experimental, which through data have revealed the devastating extent of zinc and iron deficiencies.

In the early 1970s, apparently independence of the crucial role of zinc in immunity functions for both pregnant animals and humans. According to an investigation carried out by a team of Danish investigators (Gord *et al,* 2000), prolonged zinc deficiency in pregnant woman often lead to zinc mal-absorption which resulted in foetus with several skin disorders and prone to die young of viral or fungal infections. The offspring is often found to have genetically acquired inability to absorb zinc normally through the gastrointestinal tract and a congenitally hypo plastic thymus dependent immunity function (Abassy *et al,* 1994).

Zinc deficiency in pregnancy is a frequent if not in fact invariable concomitant of protein-calorie malnutrition. Indeed, there are striking physical similarities between this syndromes produced by dietary deficiency of zinc alone (Shankar and Prasad, 1998). Also notably, the human acrodermatitis enteropathiea a scourge like disorder of young children is also inherited as an autosomal recessive trait associated with zinc mal-absorption in pregnancy. These children suffer from severe dermatologic and gastrointestinal disturbances among other symptoms and frequently die of infection in infancy.

Low level of zinc in pregnancy exerts profound and apparently specific effects upon the cellular immune function studies on the biological role of zinc in both human and animal systems have shown that, among the 90 metalloenzymes that require zinc for proper functioning are several-including thymidine kinase, DNA polymerase and DNA dependent RNA polymerase which are necessary for the synthesis of DNA and RNA. The importance of zinc and iron in growth and development is well known, they are essential for the function of several critical enzymes. While iron plays a significant role in the development and maintenance of myelin, zinc is essential for normal embryogenesis, fetal growth and protein synthesis (O'Dell, 1994).

There are several reports indicating rise in serum zinc levels during pregnancy as well as iron. Also reports on zinc however, are conflicting with some author's reporting a fall, some report no change while others report a rise. It has also been reported that zinc and iron play significant role in contributing to low birth weight. Though this role is not known, efforts are now been geared towards understanding their role in fetal outcome. The symptoms found in pregnant women at their first trimester could be a pointer to the fact that zinc level increases. Mayes (1996) reported in his studies of nutrition in pregnancy that zinc toxicity is associated with gastrointestinal irritation and vomiting. This goes accordingly with increased level of zinc. This level is sustained until delivery.

According to the United Nations Population Fund each year more than half a million women die of pregnancy related causes. In addition, the United Nations Children Fund (UNICEF) noted that more than 60 million women suffer acute complications from pregnancy annually and that nearly a third of them sustain life long injuries or infections. Of these complications, the deficiency of zinc and iron could not

be ruled out. This could be supported by the fact that most women lost so much blood during delivery and some with reduced level of iron suffer from anaemia. The risk of Spinal bifida, caused by a defective closing of the neural tube, is greatly reduced when the expectant mother has an ample supply of zinc. Since the embryo's neural tube closes between the 24th and 28th day after conception long before many women realize that they are pregnant. Some women who are planning to become pregnant are advised to take zinc supplement as well as iron supplement.

Iron is a crucial nutrient. Indeed, a woman's iron requirements double during pregnancy. If her reserve is low which is true of many women in developing countries, she can become iron-deficient or suffer from iron-deficiency anaemia. This condition can be worsened by repeated pregnancies, as the woman may not have time between them to replenish her iron reserve. In situation like this, anaemia in pregnancy is imperative (UNICEF, 2000). There are many studies reporting an alteration in plasma zinc and iron levels in pregnancy. Zinc and iron have been found to decrease in pregnancy. Zinc in particular is found to decrease as pregnancy progresses to term. For iron, its increased utilization by the developing foetus and placenta, as well as blood volume expansion, significantly increase the iron requirements during pregnancy, in essence there is a decrease level of iron in pregnancy (Bogden, 1996).

It must also be stated that both zinc and iron irrespective of their level in the pregnant and non-pregnant women, they are required in trace amounts, less than 100mg/day since they are micronutrients. Pregnant women should therefore endeavour to take diet rich enough to supply enough energy as well as the micronutrients especially zinc and iron.

Zinc levels in pregnancy

Zinc is very important in the growth and development of the foetus. It is also essential for embryogenesis and protein synthesis. Exploring the effects of zinc on immune functions in healthy pregnant women subjects, it was found that zinc increases the mitogenic influence on human lymphocytes exerting its greatest proliferative influence in concentrations somewhat higher than those present in normal human plasma (Schoetal *et al,* 1979). In patients with pregnancy complications, the proliferative responses of peripheral blood lymphocytes to zinc were depressed to the same extent as to the phytomitogen PHA. Response to zinc stimulation would appear to be adaptable as a reasonably sensitive method for the in vitro immunologic evaluation of patients with pregnancy complications (Goksu and Ozsoylu, 1986). According to Kirksey *et al* (1994), plasma zinc level decreases as pregnancy progresses. However, the moment a woman conceives the micronutrient level of zinc increases with increased metabolism in both the mother and the foetus.

Zimmerman *et al* (1993), observed a decreased both in the serum zinc and when expressed as per unit of albumin between 1st and 3rd trimester. This was attributed to hemodilution due to expansion in the plasma volume to the extent of 30% of the non-pregnant value. It was also suggested that the decrease in zinc per unit of an albumin most likely reflected an increase in zinc uptake by the placenta and foetus. Unlike earlier reported cases, no increase in maternal plasma zinc was observed at term in this study. There was actually a decrease in the zinc level in pregnancy to the extent of 20% as compared to non-pregnant levels.

Plasma zinc levels

Zinc is indispensable in the proper maintenance of pregnancy. Zinc acts as cofactor of many enzymes such as lactate dehydrogenase, alkaline phosphatase, carbonic anhydrase, etc., and the activities of these enzymes are raised during pregnancy. Therefore, there should be surge in the dietary intake of zinc to meet up with the increased metabolic activities in pregnancy. In normal pregnancies and in the three periods of the third trimester, zinc concentration in plasma of the mother is significantly lower than in non-pregnant women. Zinc concentration was mildly low (90% of the values in non-pregnant women) and remained stable during the 3rd trimester of pregnancy.

According to Reyes *et al* (2000), in normal pregnancies studied in 1994-1995, zinc levels showed no difference compared to the level in non-pregnant women. An increased in the level of zinc has also been reported earlier (Tamura *et al,* 2000). With this variability in the level of zinc in pregnant women, it means there are other things to it, probably environmental (Stadtman, 1997). Zinc levels showed a progressive decrease till term from a non-pregnant normal level of 78.1 ± 21.85 µg/dl to 60.5 ± 14.42 µg/dl, a 20 % decrease in normal zinc level as compared to the levels in control women of similar gestational ages (Yasoghara *et al,* 1991) as shown in table 1.

Table 1: Plasma zinc levels in normal pregnancy

Subjects	No	Zinc (µg/dl)
Non pregnant	15	78.1 ± 21.85
Pregnant		
≤ 12 weeks	27	85.0 ± 21.02
13-20 weeks	30	83.2 ± 24.44
21-28 weeks	44	72.7 ± 23.44
29-36 weeks	33	67.8 ± 19.52
≥ 37 weeks	27	60.5 ± 14.42

Source: Yasoghara *et al.,* 1991

Maternal and cord blood zinc levels

It has earlier been reported that cord plasma zinc levels are 50% higher than maternal plasma zinc levels (Caulfield *et al,* 1998). This has been attributed to active transport of the mineral across the placenta (Garg *et al,* 1993). The maternal and cord plasma levels of zinc were within the reported range (Yasoghara *et al,* 1991). However, the cord levels were higher in low birth weight groups with lower maternal levels contrary to other report where infants with low birth weight had either a significantly lower or no change in the cord zinc levels. While cord blood levels of most other micronutrients were significantly lower, zinc levels in cord blood are significantly higher than in the mother levels. Analysis of data with respect to birth weight suggested that maternal and cord levels of other micronutrients showed a progressive decrease with increasing birth weight. Zinc levels in cord blood plasma were significantly higher in infants with low birth weight, while levels in their mothers were lower as shown in table 2 below: this was reported by Yasoghara and his team (1991).

Table 2: Maternal and cord blood zinc levels

	Zinc (µg/dl)	
Birth weight (kg)	Maternal	Cord
≤ 2.5	64.4 ± 14.51	101.0 ± 19.42
2.51 – 3.0	63.4 ± 12.20	89.1 ± 21.26
≥ 3.1	69.8 ± 14.91	95.3 ± 19.75
Pooled	65.2 ± 13.78	94.5 ± 20.77

Source: Yasoghara, 1991

A significant correlation was seen between maternal and cord zinc levels. No correlation was observed between birth weight and zinc levels in maternal plasma. However, it was observed that 15% and 11% of mothers giving birth to infants weighing ≤ 2.5 and 2.5-3.0 kg respectively had zinc levels of 50µg/dl. No relationship could be observed between maternal age, parity and body weight on one hand and plasma zinc levels on the other (Yasoghara *et al*, 1991).

Zinc level and pregnancy induced complications

Information on the changes in the profile of these minerals (zinc and iron) in pre-eclampsia, eclampsia, pregnancy induced hypertension and intrahepatic cholestasis of pregnancy is quite limited. Intrahepatic cholestasis of pregnancy is a rare disorder of late pregnancy, characterized by skin pruritus and mild biochemical cholestasis appearing during the third trimester of pregnancy and disappearing shortly after delivery, with a tendency to reoccur in future pregnancies. Its clinical relevance is related to an increased risk of premature deliveries with a subsequent increase in prenatal morbidity and mortality and also to a high risk of stillbirths (Reyes *et al,* 2000). The levels of zinc in these complications were analysed and the result obtained are as shown in table 3. This is in comparison to non-pregnant and healthy pregnant women. For intrahepatic cholestasis of pregnancy, zinc levels were observed to remain the same throughout gestation period.

In pregnancy induced hypertension, a significant decrease in plasma copper is observed as compared to the values in normal pregnant women of similar gestational age (Table 4). In eclampsia, plasma copper levels though higher than in pregnancy induced hypertension, were not significantly different from normal. In pregnancy induced hypertension and eclamptics, low plasma zinc levels were observed as compared to the levels in normal pregnant women of similar gestation period. The reported values for plasma zinc in pregnancy induced by hypertension are equivocal with some reporting a decrease in the levels while others did not find any difference between the pre-eclamptics and normal. Shah and Sachdev (2006) reported lower zinc levels early in pregnancy in subjects who developed pregnancy induced hypertension at term. It was also reported that teenage pregnant woman experiencing hypertension had lower plasma zinc level as compared to the normal pregnant women. A decrease in zinc and an increase in copper in placentas of pre-eclamptics had been reported by Kirksey *et al* (1994), and they attributed this to changes in physiologic response (normally seen in pregnancy) which get exaggerated in pre-eclampsia. However, in pregnancy induced hypertension and eclampsia, a cause and effect relationship needs to be established between low zinc levels and the disease process.

Table 3: Serum levels of zinc in intrahepatic cholestasis pregnancy (ICP)

	Non-pregnant women	Normal pregnancies			ICP		
		27 - 32 WK	33 -35WK	> 36WK	27 - 32WKS	33 - 35Wk	> 36WKS
Zn	19.71 ± 3.21	16.21 ± 5.10	16.84 ± 5.21	17.90 ± 3.61	15.62 ± 1.60	15.57± 1.80	15.88 ± 1.92

Source: Reyes *et al.,* 2000.

Table 4: Zinc levels in normal pregnancy, pregnancy induced hypertension and eclampsia

Subject	**No**	**Zinc (µg/dl)**
Non pregnant	15	78.1± 21.85
Pregnant		
≥ 37 weeks	27	60.5 ± 14.42
Pregnancy induced hypertension	19	55.5 ± 11.02
Eclampsia	07	53.3 ± 10.01

Source: Shah and Sachdev, 2006

Effects of zinc levels in pregnancy

As reported that micronutrients are required in proportion less than 100 mg/day, the effect of low level of zinc could not be devastating if supplemented in time, but if left untreated for long, it could lead to hypogonadism, growth failure, low birth weight, impaired wound healing, decreased taste and smell activity; secondary to acrodermatitis, enteropathica and parenteral nutrition.

Iron level in pregnancy

Iron is a micronutrient that is required in trace quantity for the normal functioning of the system. It is especially needed to be supplemented in pregnancy in order to avert anaemia because the iron requirement by a pregnant woman is doubled compare to a non-pregnant woman. During pregnancy, a woman needs addition iron to satisfy the demands of the foetus, the placenta, and her increasing hemoglobin mass. Generally, there are altered nutritional requirements and drug responses. These conditions affect the nutrient in question and effort must be geared towards its management or correction.

In pregnancy: increased iron utilization by the developing fetus and placenta, as well as blood volume expansion significantly, increases the iron requirement during pregnancy (School and Hediger, 1994). The association in the use of blood in chronic bleeding and in growing pregnancy suggests a relationship in term of iron requirements. Chronic bleeding or acute blood loss may result in iron deficiency. One milliliter of blood with hemoglobin concentration of 150g/l contains 0.5mg of iron. Thus, chronic loss of very small amounts of blood may result in iron deficiency. Individuals who donate blood frequently, especially menstruating women, may need to increase their iron intake to prevent deficiency because each 500ml of blood donated contains between 200 and 250mg of iron (Jacob *et al*, 2001). The most recent RDA for iron was released in 2001 (Table 5). It is based on the prevention of iron deficiency and maintenance of adequate iron stores in individuals eating a mixed diet including the pregnant women (Maret and Sandstead, 2006). As it can be deduced above, a pregnant woman needs additional iron to satisfy her own increasing hemoglobin (Figure 1) mass etc. The placenta and the foetus contain approximately 300mg of elemental iron at birth. In addition, the maternal increase in mass accounts for about 500mg of elemental iron. Thus, the total antepartum iron requirements are 800mg (Shaw, 2001). Most of this iron is needed during the 2nd half of the pregnancy, at an approximate rate of 5.7mg daily during the last 40 days. However, because about 1mg of iron is excreted daily, the total daily need is almost 7mg daily during the second half of pregnancy. Most women of childbearing age cannot mobilize this much iron and supplemental iron must be given to prevent iron deficiency. The usual iron supplement, ferrous sulphate, contains 20% elemental iron. Thus, a 325mg tablet contains about 65mg of elemental iron, of which 10 to 20% will be absorbed. Most prenatal vitamins contain 60-65mg of iron and these should be adequate for a healthy pregnant woman. If iron stores have been depleted by poor dietary habits, recent childbirth, or other causes, however, additional iron supplementation may be necessary (Shaw, 2001).

Table 5: Recommended Dietary Allowance, (RDA 2001)

Age and life stage group	RDA for males (mg/day)	RDA for female (mg/day)
0-6 months	0.27	0.27
7-12 months	11	11
1-3 years	7	7
4-8 years	10	10
9-13years	8	8
14-18 years	11	15
19-50	8	18
51 years and other	8	8
Pregnancy all ages	-	27
Breast feeding: 18 years and younger	-	10
Breast feeding: 19 years and older	-	9

Source: Maret and Sandstead, 2006.

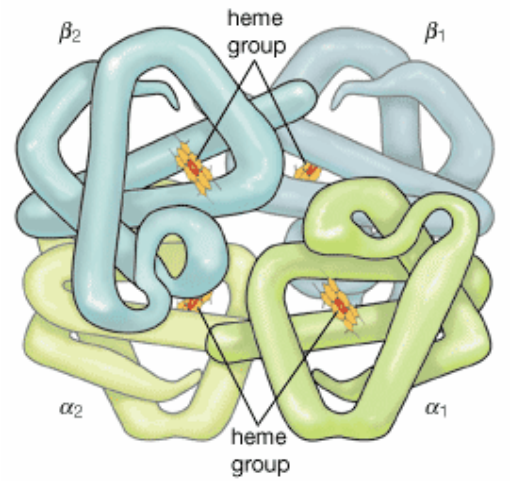

Figure 1: Molecular tetrameric structure of hemoglobin with iron (heme) binding sites (Encyclopaedia Britannica Inc., 2007)

Iron levels and pregnancy associated complications

A well balanced diet along with plenty of clean, fresh water is essential for proper pregnant women nutrition and for proper foetal growth. The deficiency of iron could lead to deficient hemoglobin (oxygen carrier in red blood cells), pica (cravings for certain foods), laziness and fatigue, heart palpitations, memory deficits and anaemia (McIntosh, 2002) epidemiologic studies provide strong evidence of an association between severe anaemia in pregnant women and adverse pregnancy outcomes, such as low birth weight, premature birth, and maternal mortality. Iron deficiency can be a major contributory factor to severe anaemia but evidence that iron deficiency anemia is a causal factor in poor pregnancy outcomes is still lacking (McIntosh, 2002). Nevertheless, most experts consider the control of maternal anaemia to be an important part of prenatal care. Elevated hemoglobin, especially in later pregnancy, is also associated with

poor pregnancy outcomes, but there is no evidence that this association is related to high iron intakes or iron supplementation. Rather, elevated hemoglobin in pregnancy is more likely to be explained by underlying conditions like pregnancy induced hypertension and preeclampsia, which are well known to contribute to poor pregnancy outcomes (Galli and Barbui, 1999).

Iron deficiency anaemia

Inadequate iron stores in the blood cause iron deficiency anaemia. Iron is needed for the production of hemoglobin needed for red blood cells. Women are more likely to be anaemic because of poor eating habits and blood loss during menstruation. Thus, many women enter pregnancy with reduced iron stores or some level of anaemia. According to multiple studies on anemia and pregnancy outcomes, anaemia, which is diagnosed early in pregnancy, is associated with increased risks of low birth weight and preterm delivery.

Pregnancy increases the risk of anaemia. During pregnancy, the blood volume expands by almost 50%. Most of the increased blood volume is an increase in blood plasma levels, not red blood cells. Since the plasma level increases more rapidly than the red blood cells in the first half of pregnancy, the concentration of red blood cells (Figure 2) in the blood is less than during the pre-pregnancy period. At least, 20% of all pregnant women are anaemic. However, this type of anemia, called hemodilutional, does not increase the risk of low birth weight or preterm delivery. In most cases, women are not aware that they are anaemic. In more severe cases, a woman may experience extreme weakness, pallor, fainting and shortness of breath etc.

Since anaemia is quite prevalent in pregnant women, physicians obtain a blood sample and check for anaemia on the first prenatal visit. Since anaemia often develops during the pregnancy many practitioners recheck the blood count later in the pregnancy (Fleming *et al.,* 1974). While practitioners use supplementation widely, studies indicate that it has little impact on pregnancy outcomes. Treatment should involve supplementation with iron. The blood ferritin level should be monitored to assess the effectiveness of treatment.

Iron level and impaired immune function in pregnancy

Iron is required by most infections agents as well as by the infected host in order to mount an effective immune response. Sufficient iron especially in pregnancy is critical to several immune functions, including the differentiation and proliferation of T-lymphocytes and the generation of reactive oxygen species (ROS) by iron dependent enzymes which are used for killing pathogens. Pregnant women are susceptible to infections due to the low level of iron in pregnancy. Thus, supplemental iron will help the pregnant women to boost their immune system to fighting infectious agent. During an acute inflammatory response, serum iron levels decrease while levels of ferritin (the iron storage protein) increase, suggesting that sequestering iron from pathogens is an important host response to infection. Despite the critical functions of iron in the immune response, the relationship between iron deficiency and susceptibility to infection, especially with respect to malaria, remains controversial. High dose iron supplementation of children timing in the tropics has been associated with increased risk of clinical malaria and other infections such as pneumonia. Studies in cell culture and animal suggest that the survival of infection agents that spend part of their life cycle within the host cells, such as plasmodia (malaria) and mycobacteria (tuberculosis) may be enhanced by iron therapy. Controlled clinical studies are needed to determine the appropriate use of iron supplementation in regions where malaria is common as well as in the presence of infectious disease, such as HIV, tuberculosis, and typhoid.

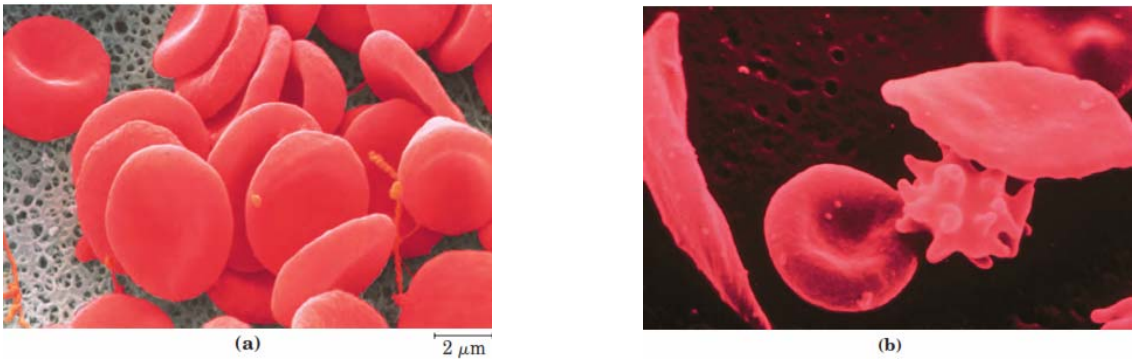

Figure 2.0: A comparison of uniform, cup-shaped, normal erythrocytes (a) with the variably shaped erythrocytes seen in sickle-cell anemia (b) which range from normal to spiny or sickle-shaped.

(Lehninger, Principle of Biochemistry, 4th Ed., p173)

Iron overload

Several genetic disorders may lead to pathological accumulation of iron in the body. Hereditary hemochromatosis results in iron overload despite normal iron intake, while sub-Saharan African hemochromatosis appears to require a combination of high iron intake and a genetic predisposition. Up to 1 in 200 individuals of northern European descent are affected by a genetic disorder known as hereditary hemochromatosis (HH). It is characterized by iron deposition in the liver and other tissues as a result of a small increase in intestinal iron absorption over many years. If untreated, tissue iron accumulation may lead to cirrhosis of the liver, diabetes, heart muscle damage (carchomyopathy), arthritis. HH was known to be a genetic disorder affecting intestinal iron absorption for many years, but the gene (HFE) and the mutation resulting in HH were only recently identified in 1996. At present, the exact role of protein encoded by the HFE gene in intestinal iron absorption is not well understood (Gidon-Jeangirard, 1999). Iron overload in HH is treated by phlebotomy, the removal of 500ml of blood at a time, at interval determined by severity of the iron overload. Individuals with HH are advised to avoid supplemental iron, but are not generally advised to avoid iron-rich foods. Alcohol consumption is strongly discouraged due to the increased risk of cirrhosis of the liver. The explanation goes for the pregnant women too and therefore proper medical attention should be sought before iron supplementation is embarked upon (Shaw, 2001).

African hemochromatosis (iron overload: Iron overload in black South African pregnant women is associate with chronic exposure to diets containing too much iron derived mainly from cooking pots and steel barrels used to ferment beer. This form of iron overload is usually more severe in adult men, whose beer consumption tends to be higher and whose iron intake may exceed 100mg/day. Like HH it may also result in liver cirrhosis and diabetes. Unlike HH, African hemochromatosis appears to require high iron intake in association with a genetic factor that has not yet been identified (Shaw, 2001).

Conclusion

The influence of nutrition, especially adequate supply of zinc and iron in pregnancy, on the health, behaviour and well being of pregnant women cannot be overstated. Good and adequate supply of zinc and iron including the resultant deficiencies have been discussed and this is the only way to ensure the growth, health, activity of the foetus, reproduction by the mother and a disease resistant offspring. With this review, one would see that the serum iron test is essential because of its benefit which is a constant baseline of nutrition helping to control unwanted nutritionally induced variables. In addition, regular test is required in pregnancy to ascertain the nutritional status of the woman to reduce further the possibility of those unwanted variables listed earlier on. Experts and physicians also need to improve in their nutritional habits and to eat food rich in zinc and iron.

At this juncture, the emphasis need to be laid on the interactions between this two elements, their interactions with the food components and interactions of each with other nutrients. It is as simple as intuitively adding a dietary supplemental programme. Mixtures of vitamins and minerals can cause extensive morbidity, particularly the use of vitamin C in large doses. Magnesium salts cause purgation and further ions of essential metals such as zinc. Do-it-yourself supplementation can get out of hand. For example, in children with autism, there is already extensive disruption of biochemistry, so blindly adding supplements on a prospective basis can exacerbate the illness. Though a pregnant woman iron requirements increased, this should not mean that the serum level should not be determined before a supplementation is taken up. And as put forward by Shaw, the total requirement is 800mg; supplementation should therefore be based on this except there is a regular blood loss or donation of blood. This is imperative in order to avoid iron overload and zinc overload.

Specific metals which are in deficiency should be replaced, but the use of complex mixtures of metal can be often self defeating. For example zinc mixed with copper and iron gives a competitive mixture for absorption in the gut. The actual absorption of any one of these will be at best erratic and result in wastage due to non-absorption. Many high street preparations contain this mixture. There has to be a safety aspect of this, zinc given incorrectly in pregnancy can lead to copper or iron deficiency, the symptoms of which are unpleasant and can be puzzling to investigating physicians who are unaware of dietary supplementation.

The best recommendation for supplementation is the usual one, ferrous sulphate, this is because it contains 20% elemental iron and a 325mg tablet will be okay. The effect of zinc deficiency also cannot be overemphasized, a lot of important enzymes are zinc dependent among which are thymidine kinase, DNA polymerase and other enzyme necessary for the synthesis of DNA and RNA. Thus, the deficiency or low level of zinc in pregnancy will have a profound effect on the mother as well as the foetus; the human acrodermatitis enteropathic is also a reflection of zinc deficiency in pregnancy.

Interactively, vitamin A deficiency may exacerbate iron deficiency anaemia in pregnancy. Vitamin supplementation has been shown to have beneficial effects on iron deficiency anaemia and improve iron status among children and pregnant women. The combination of vitamin A and iron seems to reduce anemia more effectively than either iron or vitamin A alone. Animal studies demonstrate a role for copper in iron absorption and iron has been found to accumulate in the livers of copper deficient animals,

indicating that copper is required for iron transport to the bone marrow for red blood cell formation.

Iron and zinc if taken together in high doses on an empty stomach can cause inhibition of zinc absorption. Iron fortified foods have no effect on zinc absorption. Iron when consumed with calcium in a single meal, calcium has been found to reduce the absorption of iron. However, little effect has been observed on serum ferritin levels with calcium supplement levels ranging from 1,000 to 1,500 mg/day.

In conclusion, it has been established that both zinc and iron are very useful in pregnant women and their requirements increase with pregnancy. Their side effect that is deficiencies are costly and destructive, all pregnant women therefore need to be harmed with this information so that they can avert any complication that may arise from this. Efforts should be geared towards prenatal, antennal and postnatal clinic to know the zinc and iron level in the system so as to avoid any unforeseen circumstances and complications associated with these conditions.

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Effect of storage on rheological and sensory characteristics of cow and buffalo milk yogurt

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Abstract

Milk is complete diet and essential nutritive food for all age groups especially children. Yogurt is rich in protein, several B vitamins and essential minerals. Knowledge of its rheological properties is of primary importance in processing, handling, process design and quality control in product development especially yogurt. Yogurt is delicious and health promoting dairy product due to its therapeutic properties and high nutritional value. In Pakistan yogurt is daily consumed in the form curd, dahi and packed yogurt. Yogurt was prepared from cow and buffalo milk and compared for rheological and sensorial characters during storage. Means for viscosity (6169-3701cp), flavor (37.78-14.56), texture (24.67-13.00), appearance (11.00-4.44) and taste (6.94-3.61) decreased while firmness (14.11-15.22) increased during storage. Buffalo milk yogurt showed more viscosity (8944) and firmness (20.17) than cow milk yogurt (2339, 10.08 viscosity and firmness respectively). Yogurt from cow milk got higher score for texture (19.00) and taste (5.87) than buffalo yogurt (17.50, 4.25 texture and taste respectively) while flavor (26.91) and appearance (7.75) of buffalo was better than that of cow milk yogurt (25.91, 6.67 for texture and appearance respectively). On overall basis yogurt made from buffalo milk showed better rheological and sensory characteristics and liked by the panel of judges.

Key Words: Milk, composition, yogurt, rheology, sensory, storage

Introduction

Pakistan is 4th largest milk producing country in the world. Milk production was 46.440 million tons of during 2010-11 (GOP, 2010-11). Pakistan is 2nd largest buffalo-milk producing country in the world (Bilal *et al*., 2008). Higher concentration of nutrients in buffalo milk makes it interesting for researchers and investors in various countries (Amarjit and Toshihiko, 2003). Buffalo milk contains higher contents of fat, protein, lactose, total solids, vitamins and minerals than cow milk which imparts a rich flavor and taste. The excessive concentration of nutrients makes buffalo milk a highly suitable ingredient for the manufacturing of a wide range of products including cheese, butter, ice cream and yogurt (Fundora *et al*., 2001).

Yoghurt is a fermented dairy product obtained by lactic acid fermentation of milk by the action of yoghurt starter bacteria (Köse and Ocak, 2011). It is more nutritive than milk due to higher milk solids, protein contents, calcium, phosphorus and a range of vitamins in addition to nutrients developed during fermentation (Patel, 2011; Fadela *et al*., 2009). Levels of some vitamins, such as vitamin B_1 and pantothenic acid are reduced as they are utilized by the bacterial culture. Flavor and consistency are its main quality parameters in yoghurt (Vercet *et al*., 2002). Consumer acceptance of yogurts is based on physical attributes like lack of syneresis and perceived viscosity (Lee1 and Lucey, 2010), acidity and aroma perceptions and the textural properties (Penna *et al*., 2006; Béal *et al*,. 1999) crucial for the quality and overall sensory performance. Viscous properties are of primary importance with respect to the quality of the products (Magenis *et al*., 2006). Yogurt has non-Newtonian flow properties with strong time dependence on both the thixotropic and viscoelastic types (De Lorenzi *et al*., 1995). Di Cagno *et al*,. (2004) noticed pseudoplastic behaviour in fermented milks made from mare's milk.

Rheology is a science related to the flow of fluids and deformation of matter (Steffe, 1992). Rheological properties of yogurt during the preparation are affected by different sources of milk (Jumah *et al*., 2001). Viscosity of yogurt is affected by total solids, milk composition, homogenization, type of culture, acidity, stabilizer, degree of proteolysis and preheat treatment of milk (Kalab *et al*., 1983; Heertje *et al*., 1985; Harwalkar and Kalab, 1986; Biliaderis *et al*., 1992). Viscosity and firmness of yogurt increase with increase in total solid content of milk (Becker and Puhan, 1989). The properties of yogurt such as: titratable acidity, pH, free fatty acids contents, aromatic compounds (acetaldehyde, diacetyl

and ethanol), sensory quality and nutritional value depend on the different factors including quality of milk used to the production, processing technology, food additives and kind of yogurt starter culture and its activity. Fermentative, aromatic, lipolytic and proteolytic ability of starter bacteria vary that ultimately effect the viscosity of the product (Tamime and Robinson, 1999).

Rheological properties of yogurt are complex and influenced by solid content, physical states of fats and proteins in milk, milk composition, temperature and time of milk heat pretreatment, mechanical handling of coagulum, use of stabilizers, type and quantity of starter culture incorporated for inoculation (Paskov *et al*., 2010), homogenization, acidity, degree of proteolysis and heat pretreatment of milk (Hirano *et al*., 1998), fermentation temperature and storage conditions of the final product (Sendra *et al*., 2010). Correlation among yogurt rheology and structure have been investigated by many scientists employing a variety of techniques and instruments for evaluating the effect of milk heat treatment, type of starter culture, incubation temperature, storage time (Girard and Schaffer-Lequart, 2007; Ozer *et al*., 1997; Remeuf *et al*., 2003; Renan *et al*., 2009; Sodini *et al*., 2005). The textural differences between the yogurts are attributed to the kind of milk used and their compositional differences. Texture is a critical aspect of consumer acceptability of yogurt (Muir and Hunter, 1992; Cobos *et al*., 1995). Rheological properties influence texture affecting sensory perception and ultimately the acceptance of a product by the consumer (Aichinger *et al*., 2003).

Understanding the rheological behavior of dairy products is important in quality control of both ingredients and finished products, design and evaluation of processing equipment, unit operations and process parameters, adjustment of time x temperature x flow rate selection of fluid dairy products and characterization and development of dairy products for consumer acceptability and elucidation of the relationship among structure and textural properties (Kokini, 1992; Barbosa-Canovas *et al*., 1993). It helps in predicting texture and stability of yogurt (Paskov *et al*., 2010). Knowledge of the behavior of yogurt during long storage is important because its shelf life is based on whether the products display any of the physical, chemical or sensory characteristics that are unacceptable for consumption. Studies of changes in these quality characteristics during storage would enable producers to predict the shelf life of the product more accurately (Salvador and Fiszman, 2004). Study was planned to compare rheological properties of yogurt prepared from cow and buffalo milk and to study the effect of storage time on the rheological and sensorial properties of yogurt prepared from cow and buffalo milk.

Materials and Methods

Procurement of raw material

Fresh milk of cow and buffalo was obtained from livestock Farm of University of Agriculture Faisalabad. Milk was filtered through a muslin cloth.

Yogurt production

Yogurt was prepared according to the procedure described by Stelio and Emmanuel (2004) using cow and buffalo milk. The inoculated milk was poured in cups of 400 mL volume, labeled and incubated at 45°C for about 3 hours resulting in 0.8-0.9% lactic acid.

Storage

The yoghurt was cooled to a temperature of 4-6°C to check further fermentation and analysis during storage.

Rheological Analysis of Yogurt

Viscosity was determined by using a Brookfield (LVDVE 230) viscometer following Gassem and Frank (1991). Firmness of yogurt gel was measured in situ in the fermenting container by texture profile analysis following method developed by Breene (1975) as described by Hirano *et al*., (1998) using rheometer with a cylinder plunger (16 mm), at compression rate of 5 mm sec^{-1} and 75% (22 mm) deformation at 10°C.

Sensory Evaluation

Texture, appearance and flavor was evaluated by the method describe by (Bonczar *et al*., 2002).

Statistical analysis

The data obtained was analyzed by using completely Randomized Design (CRD) and the means was compared by Duncan's Multiple Range (DMR) test as described by (Steel *et al*., 1997).

Result and Discussion

The yogurt samples were stored at 4-6ºC and evaluated rheologically and organoleptically at 0, 5, 10 and 15 days of refrigerated storage respectively.

Rheological characteristics

In set-type yogurt textural characteristics of curd are primary interest, while flow behavior is given more emphasis in stirred type-yogurt (Park *et al*., 2005).

Viscosity

The viscosity of yogurt decreased gradually during storage. Yogurt prepared from buffalo milk got maximum mean (12140 cp) for viscosity at day 0 while minimum mean value (1920 cp) was observed in cow milk yogurt at 15^{th} day (Fig. I). Treatment mean was maximum (8944cp) in buffalo milk yogurt while lowest in cow yogurt (2339cp) (Table II).

Table 1. Effect of storage on rheological characteristics of yogurt samples

Physical Properties	Storage Days			
	0 day	5 days	10 days	15 days
Viscosity	6169 a	4415 b	4178 c	3701 d
Hardness	14.11	14.67	14.78	15.22

Table 2. Effect of treatments on rheological characteristics of yogurt samples

Physical Properties	Treatments		
	Commercial yogurt	Cow milk yogurt	Buffalo milk yogurt
Viscosity	2564 b	2339 c	8944 a
Hardness	13.83 b	10.08 c	20.17 a

Table 3. Effect of storage on sensorial attributes of yogurt samples

Sensory Characteristics	Storage Days			
	0 day	5 days	10 days	15 days
Flavor	37.78 a	33.11 b	22.00 c	14.56 d
Body and texture	24.67 a	21.33 b	17.33 c	13.00 d
Taste	6.94 a	5.88 b	4.88 b	3.61 c
Appearance	11.00 a	8.33 b	6.78 c	4.44 d

Table 4. Effect of treatments on sensorial attributes of yogurt samples

Sensory Characteristics	Treatments		
	Commercial yogurt	Cow milk yogurt	Buffalo milk yogurt
Flavor	27.75 a	25.91 c	26.91 b
Body and texture	20.75 a	19.00 b	17.50 c
Taste	5.87 a	5.87 a	4.25 b
Appearance	8.50a	6.67 b	7.75 a

Highest mean value (6169cp) for storage was recorded at start of storage which decreased significantly to 3701cp at 15th day (Table I). The viscosity of yogurt decreased from 3535 to 1978cp for commercial yogurt, from 2833cp to 1920 cp for cow milk yogurt and for buffalo milk yogurt 12140cp to 7240cp after 15 days storage (Fig. I). The results of present study in accordance with (Aryana *et al*., 2006; Gassem and Frank, 1991) they reported a decrease in viscosity of yogurt with increase of storage of time. Olivera *et al*., (1996) observed that different types of starter bacteria resulted in changes in yogurt viscosities during storage, implying the role of microorganisms in affecting yogurt viscosity. Starter bacteria have proteases (Kosikowski, 1982) which act on the yogurt protein matrix over time, resulting in lower viscosities.

Firmness

The maintenance of a uniform texture and hardness among different units, processing dates and shelf life is a prime goal in yogurt production (Chanasattru *et al*,. 2002). Yogurt exhibits an irreversible time-dependent effect or "irreversible thixotropy" (Ramaswamy and Basak, 1991; Benezech and Maingonnat, 1994; Afonso and Maia, 1999). The textural differences between the yoghurts are attributed to the kind of milk used and their compositional differences. Yoghurt produced from ovine milk had a greater firmness than caprine yoghurt because it had the highest content of protein and total solids (Trachoo and Mistry, 1998; Stelios and Emmanuel, 2004).

The firmness of yogurt was not significantly affected by storage period while the results were highly significant for treatments. Mean for firmness was recorded to be highest (21.33pas) in yogurt samples from buffalo milk at day 15 while lowest (9.00pas) in cow milk yogurt at 10th day (Fig. II). Storage mean for firmness increased gradually from 14.11 pas to 15.22 pas (Table I). Buffalo milk yogurt showed gradual increment in firmness while cow and commercial yogurt samples showed no definite trend (Fig. II). Among treatments, yogurt from buffalo milk got maximum score (20.17 pas) for firmness which is significantly higher than commercial (13.83 pas) and cow (10.08 pas) (Table II). Firmness value for commercial yogurt decreased from 13 to 12 pas, for cow milk yogurt ranges from 11 to 10.33 pas and for buffalo milk yogurt it is 21.33 to 18.33 pas after 15 days storage (Fig. II). Firmness values increase gradually in all yogurt samples with increasing storage days (Fig. II).

The buffalo milk yogurt has more hardness than cow milk and commercial yogurt which is due to high protein content present in buffalo milk yogurt (Becker and Phuan, 1989). Results of present study are in line with those reported by Salvador and Fiszman (2004). Gassem and Frank (1991) found that no significant effect of storage time occurs on hardness of yogurt.

Sensory evaluation

All samples of prepared yogurts were sensually evaluated with intervals of 0, 5, 10 and 15 days for organoleptic properties.

Flavor

Significant differences were observed for mean flavor score among different storage intervals while treatments mean showed non significant variation. The mean flavor score of different yogurt samples decreased during storage in all treatments (Fig. III). Significantly higher mean value (39.00) for flavor score was recorded in T1 followed by T2 (37.33) which is statistically at par with T3 (37.00) at start of storage (0 day) while minimum value for flavor was found in T2 (13.67) which is statistically similar to T3 (14.00) at 15th day (Fig. III). The mean flavor scores decreased from 39 to 16 for commercial yogurt, 37.33 to 13.67 for cow milk yogurt and flavor score for buffalo milk decreased from 37 to 14 after 15 days of storage decreased (Fig. III). Mean values showed significant decrease (37.78 to 14.56) in flavor scores during storage indicating 61.46% decrease (Table III). Commercial yogurt samples showed highest (27.75) mean for flavor while lowest in cow milk yogurt (25.92). Flavor of buffalo milk yoghurt was slightly better than yoghurt prepared from cow milk as perceived by panelists and got slight higher mean for flavor (26.92) throughout the storage period (Table IV).

Fadela *et al*. (2009) reported a similar decreasing trend in flavor of ewe's milk yoghurt (19.5 to 15.5) in 21 days storage. The decrease in flavor is correlated with the proteolytic activity of bacteria and the production of higher acidity (Abrahamsen, 1978). Loss of flavor is attributed to fat and protein degradation (Mottar *et al*., 1979) and development of slight sharp flavor produced by coliform bacteria, clostiridiums spp. and other organisms. The results are in agreement with the findings of (Farooq and Haque, 1992; Tarakci and Kucukoner, 2003; Salwa *et al*., 2003) they found a decrease in flavor of yogurt during storage.

Body and texture

Statistical analysis revealed that the effect of treatments was highly significant while significant for storage period. Mean values for body and texture of yoghurt decreased significantly during storage irrespective of treatments (Fig. IV). T1 got maximum mean value (26.00) for body and texture at 0 day while significantly lower values was recorded in T3 (11.00) at end of storage (Fig. IV). The mean for body and texture scores decreased from 26 to 15 for commercial yogurt, 25 to 13 for cow milk yogurt and 23 to 11 for buffalo milk yogurt after 15 days of storage time (Fig. IV). Significant variation was recorded in storage means among different intervals while non significant difference in treatment means.

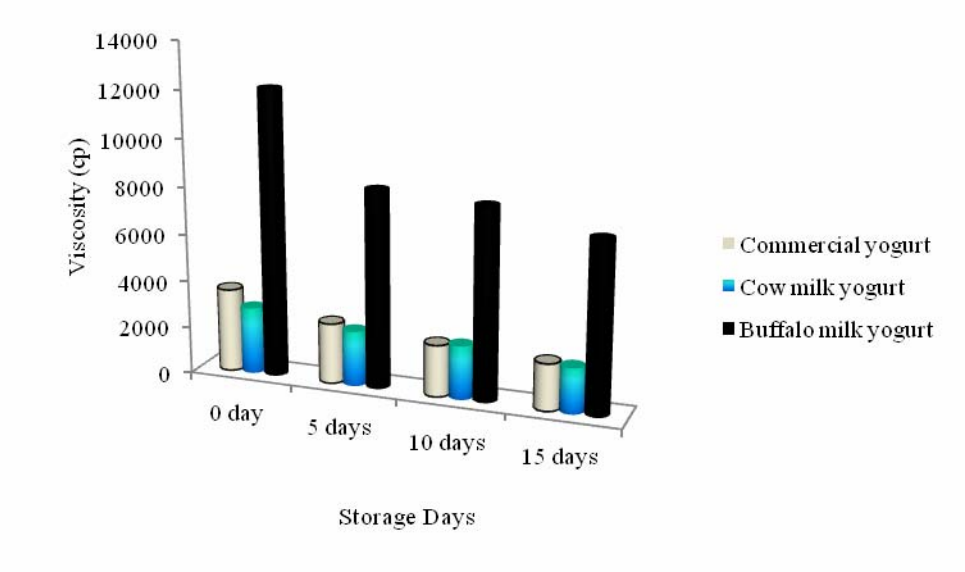

Fig.1. Individual comparison of treatment means for viscosity of different yogurts

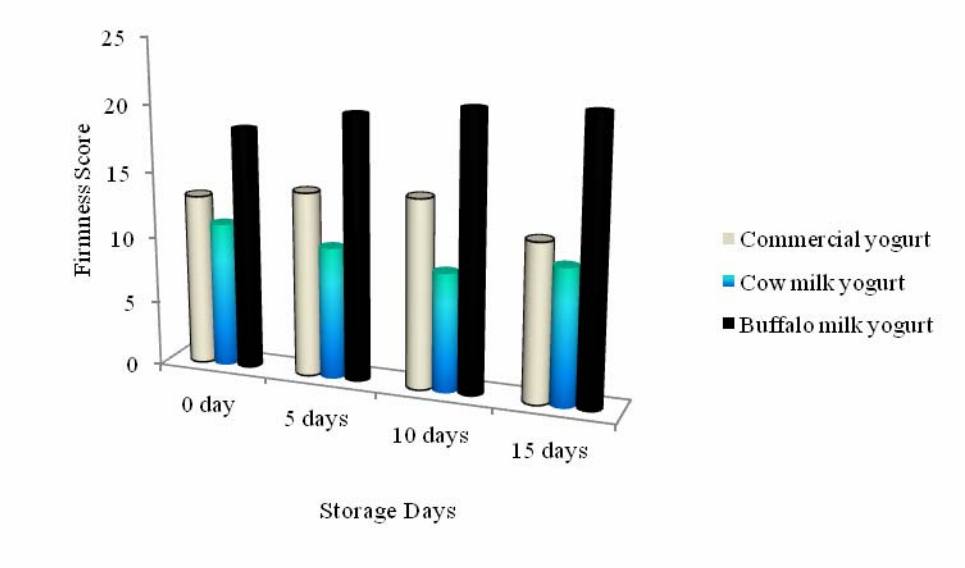

Fig.2. Individual comparison of treatment means for firmness of different yogurts

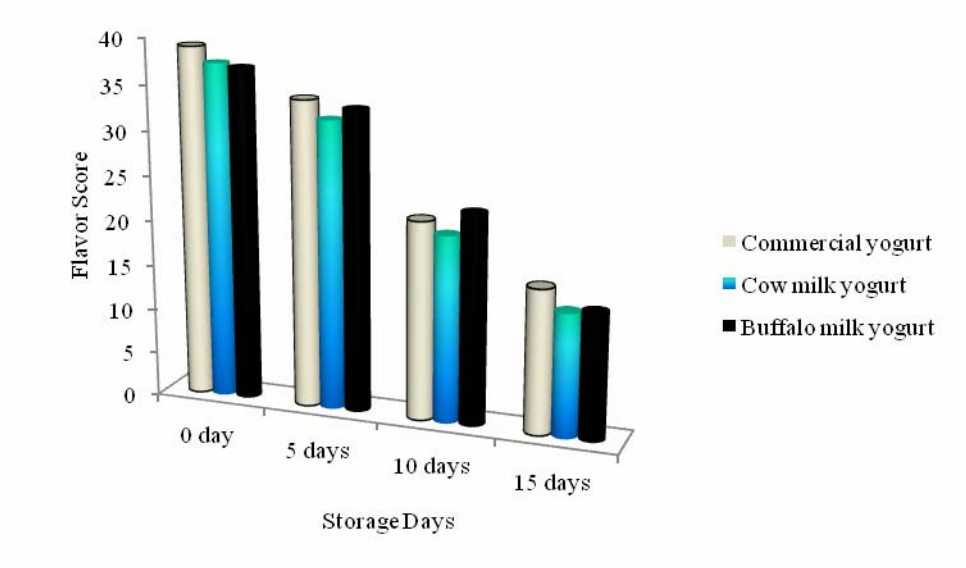

Fig.3. Individual comparison of treatment means for flavor score of different yogurts

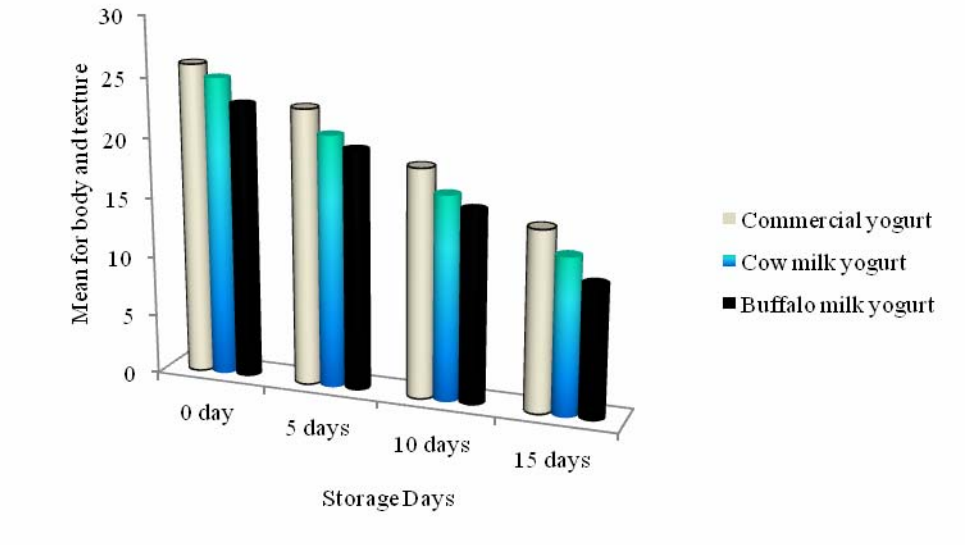

Fig.4. Individual comparison of treatment means for body and texture of different yogurts

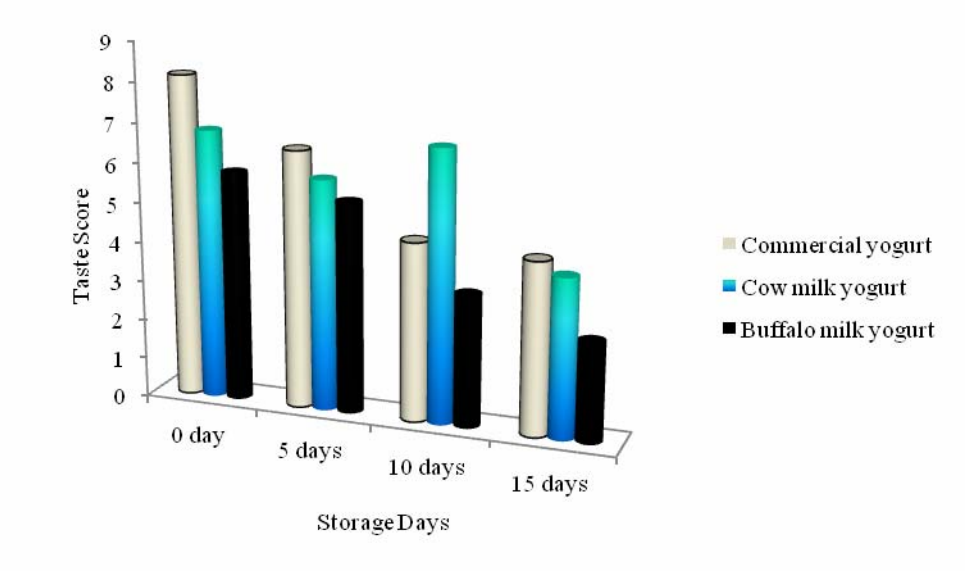

Fig.5. Individual comparison of treatment means for taste score of different yogurts

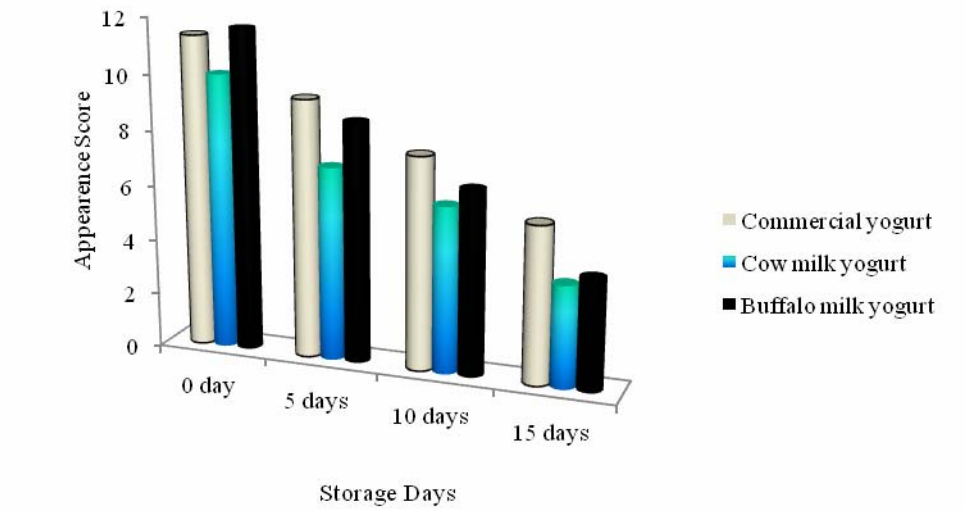

Fig.6. Individual comparison of treatment means for appearance of different yogurts

Storage means showed a gradual declining trend and value decreased from 24.67 (day 0) to 13.00 (15th day) showing 47.30% decrease over 15 days storage (Table III). Treatment mean score for body and texture was highest (20.75) for commercial yogurt while lowest (17.50) for buffalo milk yogurt (Table III). Yogurt made from cow milk showed better texture than those prepared from buffalo milk which may be due to higher SNF in cow milk than buffalo milk.

Texture acceptability increased with increasing total solids significantly (Mahdian and Tehrani, 2007) because higher TS increases gel firmness and reduce the degree of syneresis (Mohammeed *et al*., 2004). The results closely agreed with the findings of (Farooq and Haque, 1992; Tarakci and Kucukoner, 2003; Salwa *et al*., 2003) they reported a decrease in score of body and texture of yogurt during storage.

Taste

Treatments showed highly significant effect while significant in case of storage period. Storage means varied significantly while treatment means showed insignificant differences for taste. The mean taste score of

various yogurt samples decreased significantly during storage. The mean taste scores of yogurt samples decreased from 8.16 to 4.33, cow milk yogurt 6.83 to 4.00 and for buffalo milk yogurt 5.83 to 2.50 after 15 days storage. Highest mean score for taste was observed in T1 (8.16) at the start while significantly lower in T3 (2.50) at 15th day of storage (Fig. V). Commercial and cow milk yogurt got same mean value for taste (5.87) (Table IV). Cow milk yoghurt was preferred by panelists for taste and got higher scores for taste (5.87) than buffalo milk yoghurt (4.25) over entire storage period (Table IV). Decreasing trend was observed in storage means and values decreased from 6.94 to 3.61 showing 47.98% decrease in taste score among different storage intervals (Table III).

The loss of taste in yoghurt samples may be due to development of acidity, oxidation of fat or proteolysis of proteins. The results of present investigation are favored by findings of Abrahamsen (1978) who found that acidity development continued in yogurt during storage even at 3 ºC and Belomarkovic (1982) observed rapid changes in acidity at 4 ºC and noticed marked differences in taste on the second and fourth days of storage.

Appearance

Storage means showed significant variation for appearance score among different storage intervals while treatments differed non significantly. The mean scores for appearance decreased gradually during storage. The mean score for appearance decreased from 11.33 to 5.66 of commercial yogurt, 10 to 3.66 for cow milk yogurt and for buffalo milk yogurt 11.66 to 4.00 after 15 days storage. T3 exhibited significantly higher (11.67) mean value for appearance of yoghurt at start of storage while lowest mean was recorded in T2 (3.67) at end of storage (Fig. VI). Storage means showed a decline in mean values with increase in storage period and values decrease from 11.00 to 4.44 indicating 59.64% decrease in appearance score on 15th day storage period (Table III). Among treatments, commercial yogurt exhibited maximum mean value (8.50) for appearance while minimum in cow milk yogurt (6.67) (Table IV). Yogurt made from buffalo milk showed better appearance with mean value (7.75) than cow milk yogurt and preferred by panelist for appearance possibly due to higher fat content and less carotenoids in buffalo milk.

During storage the appearance of yogurt was affected and not acceptable which ultimately deteriorate the quality of yogurt. Growth of some yeasts like mould may be the reason as reported by Baraquio *et al*., (1981) that appearance of yogurt is affected due to increase in yeast and mould counts in yogurt during storage. Farooq and Haque (1992) and Tarakci and Kuckoner (2003) reported similar results and found the decrease in scores of appearance of yogurt during storage. The results are in agreement with findings of Salwa *et al*., (2003) who reported a decrease in score of appearance of yogurts during storage period.

Conclusions

Storage intervals showed significant effect on rheological and sensory characteristics of yogurts. Mean scores for viscosity, flavor, texture, appearance and taste decrease significantly with increasing storage while firmness increased. Yogurt prepared from buffalo milk yogurt showed better rheological properties (more viscosity and firmness) than cow milk yogurt. Among sensory characteristics, yogurt made from cow milk got higher score for texture and taste than buffalo yogurt while flavor and appearance of buffalo milk yogurt was better than cow milk yogurt. On overall basis yogurt made from buffalo milk showed better rheological and sensory characteristics and liked by the panel of judges.

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Osmotic dehydration technique for fruits preservation-A review

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Abstract

Fruits are important sources of vitamin and minerals. They are got rotten before the final consumption due to lack of preservation and storage facilities. Osmotic dehydration is an operation used for the partial removal of water from plant tissues by immersion in an osmotic solution. This is a useful technique to extend the shelf life and decrease the energy cost. It also helps to improve the sensorial, nutritional and organoleptic properties of foods. Along with freezing and deep fat frying make the better quality final product. It results in little bit loss of aroma in dried and semidried foods. Osmotic dehydration has become more popular in food processing industry.

Key words: Preservation, extend shelf life, dehydration

Introduction

Fruits are important sources of digestible and indigestible minerals, carbohydrates and certain vitamins, particularly vitamins A and C. The moisture in most of the fruits above 75% and fruits are prone to spoilage by molds and yeasts (Janisiewicz *et al.,* 1999). The essential amounts of vitamins, protein, minerals, dietary fiber and calories that provide the nutritional values are predictable and documented (Salunke *et al*., 1991). Fruits and vegetables are produced during peak seasons but due to lack of preservation and storage facilities, the market become overstocked during such periods and get rotten prior to reach the final consumer.

Osmotic dehydration (OD) is one of most important complementary treatment and food preservation technique in the processing of dehydrated foods, since it presents some benefits such as reducing the damage of heat to the flavor, color, inhibiting the browning of enzymes and decrease the energy costs (Alakali *et al*., 2006; Torres *et al*., 2006). Osmotic dehydration results in increased shelf-life, little bit loss of aroma in dried and semidried food stuffs, lessening the load of freezing and to freeze the food without causing unnecessary changes in texture (Petrotos and Lazarides, 2001). It has been reported that osmotic dehydration reduced up to 50% weight of fresh vegetables and fruits (Rastogi and Raghavararo, 1997).

Osmotic dehydration involves the immersion of foods (fish, vegetables, fruits and meat) in osmotic solution such as salts, alcohols, starch solutions and concentrated sugars, which some extent to dehydrates the food (Erle and Schubert, 2001). Different types of solutes such as fructose, corn syrup, glucose, sodium chloride and sucrose are used as osmotic agent for OD (Azuara and Beristain, 2002). Low molar mass saccharides (sucrose, glucose and fructose) make easy the sugar uptake due to high diffusion of molecules. It has proved to be a good quality method to get modestly processed fruits, due to the much sensory resemblance between the natural and dehydrated products (Sousa *et al*., 2003).

The influence of osmotic dehydration which has much paid attention on the solute kinetics or water swap inside and outside of food tissues (Giraldo *et al.,* 2003), the application of vacuum pulse during osmotic treatments and special sound effects on impregnation time (Mujica-Paz *et al.,* 2003). The diffusion of water is effected to natural substances such as acids, vitamins, minerals, colorants and saccharides (Lombard *et al.,* 2008). But this flow is quantitatively negligible. Osmotic dehydration which improves the sensorial and nutritional properties, preserve and improve the organoleptic properties of foods. It is efficient in room temperature. In food processing industry, osmotic dehydration has become more popular (Tortoe *et al*., 2007).

The different types of osmotic agents such as glucose, sorbitol, sucrose and salts are used according to the final products (Singh *et al.,* 2008). However combination of different solutes can be used (Taiwo *et al.,* 2003). Water loss from vegetables and fruits took place in first two hours and maximum sugar gain within 30 minutes (Conway *et al.,* 1983). Osmotic dehydration is used with other drying methods such as freezing and deep fat frying to make available better quality final product (Torreggiani and Bertolo, 2001a; Behsnilian and Speiss, 2006). Temperature and concentration of osmotic syrups increased the rate of water loss during OD. However higher temperature has the significant effect on the structure of tissues (Lazarides, 2001) and cause flavor deterioration and enzymatic browning at temperature above 45°C.

Along with osmotic dehydration, freezing is another important method. Fruit freezing can be resulted to change in color, flavor and texture of fruits as well as to maintain enzyme activity. Freezing of fruits can be resulted in decrease quality, due to poor texture and enzymatic browning action (Tregunno and Goff, 1996). The frozen products can be stored at temperature of -29°C in Europe to maintain the quality of frozen products. At a temperature of -18°C or lower can stop the products from microbiological damages, if the temperature fluctuation is not wide (Buckle *et al.,*

1987). Freezing and frozen storage cause the chemical and physical changes which lead to loss of quality. The main advantage of osmo-dehydrofreezing is not only economical but save energy, packaging and cost of distribution due to importance of the product. The dehydrfreezing process also concerned with quality improvement (Raoult-Wack, 1994).

The outcome of frozen storage is the regular increasing and permanent quality loss occurred with time (Martinez-Monzo *et al*., 2001). Freezing damage is the result of different type of processes including dehydration damage, solute concentration damage and mechanical damage caused from ice crystals (Reid, 1993). Pre-freeze treatments of dehydration were used to avoid the cellular structure loss, that caused by chemical and physical measures of freezing the fruit tissues (Huxon, 1982). Freezing has stabilizing effect, other steps of fractional dehydration allowed nutritional, sensory, structural and functional characteristics of raw material to be customized (Torregianni and Bertolo, 2001).

History of osmotic dehydration

Pointing and coworkers in 1966 were pioneering the research on OD of foods (Pointing *et al*., 1966), and since after that a continuous stream of publication was appeared (Rastogi *et al*., 2002). By using of osmosis process 50% of original weight of fruit was reduced, after that it was subjected to freeze or vacuum dried. Monograph of apple was calculated the drying rate of osmotic dehydration provided by Farkas and Lazar (1969). Vial *et al.* (1991) and Heng (1990) studied the OD (kinetics) of papaya and kiwi in sucrose and glucose solutions.

The constancy of osmotically processed cherry studied by Torregianni *et al.* (1987), to analyze the sugar content, color, acidity, vitamin C, pH and organoleptic distinctiveness. The transfer of mass during OD of pineapple was reported by Beristain *et al.* (1990). Many research papers or review papers were published (Torregianni, 1993) dealing with a variety of parameters, such OD mechanism and modeling of solid gain and water loss (Rastogi *et al*., 2002).

Osmotic dehydration of fruits

In modern period, osmotic dehydration is an important intermediate step or pretreatment technology which got much attention in the field of preservation of fruit such as reducing the consumption of energy and improved the food products quality. Much of work has been done on the kinetics of solute /exchange of water in and out of food tissues (Giraldo *et al.,* 2003), application of vacuum pulse during osmotic treatments and special effects on impregnation time (Mujica-Paz *et al.,* 2002).

Osmotic dehydration (OD) was most popular method of pretreatment drying for food materials which caused the reduction of energy costs and better the quality of end products (Andres *et al*., 2007; Ortega- Rivas, 2007). OD was frequently done by immersing the sample in concentrated solutions of salt or sugar. It was to apply in variety of fruits by decreasing the moisture contents up to 30% (Rastogi *et al*., 2002; Beaudry *et al*., 2004). The potential of chemical that existed between solution and sample of food which led to transfer mass fluxes, that's why water come out of the sample and solutes entered in to tissue. Since osmotic pressure was dynamic force for transfer of mass, OD was time consuming and slow process (Dhingra *et al*., 2008; Pardo and Leiva, 2009). As a result, to change the formulation of food system and enabled for further processing (Torregianni and Bertolo, 2001).

In sucrose solution, the osmotic dehydration of mango subjected by temperature (30–50°C), immersion time (60–150 min) and concentration of solution (40–60% w⁄ w) was studied. The water loss was maximum and incorporation of solid was minimum to get the product similarity with non processed fruit. Removal of water up to 25% with less than 6% solid uptake could be possible if condition was suitable by using sucrose 44% (w ⁄ w) solution, temperatures (38°C) and processing time up to 80 min (Azoabell and Francinaide, 2008).

Osmotic dehydration of Andes berry and tamarillo by using of three different osmotic agents: sucrose (70%), sucrose (70%)-glycerol (65%) 1:1 and ethanol. Water activity in fruits was lowered and promoted the constituents of flavor and moving of anthocyanins to somotic solution by using of this practice (Osorio *et al*., 2007). The loss of water and solid gain was caused by the application of osmotic treatments. The most helpful effect of osmotic dehydration was on lycopene, ascorbic acid and on the color quality. The result of osmotic pretreatment enhanced constancy of frozen product and extended shelf life (Olatidoye *et al.,* 2010).

The pigments, flavor precursors and volatile compounds were transferred from fruit to osmotic solution. It was suggested that osmotic syrups can be effectively applied to natural additives in food and pharmaceutical industry (Morales *et al*., 2005). Mango slices were applied to osmotic dehydration in different hypertonic solutions of sucrose and glucose at three different temperatures (30, 50 and 60°C) to devoid of agitation (Ngoran Essan Bla Zita *et al.,* 2009).

The mechanical response of mango was studied by using of 45 and 65°Brix as osmotic treatments which consisted of calcium lactate at different concentrations (0%, 1% and 2%), at the start of process, the vacuum pulse was applied. Dehydrated mango samples at 30°Brix were characterized as mechanical properties such as sugar and gain of calcium, loss of water and changes were done during treatments. Through compression test, mechanical properties were measured which effected by treatment conditions. At 2% concentration of osmotic solutions was influenced of calcium on mechanical properties by using 45°Brix of sucrose and vacuum pulse and promoted the calcium and solute gain. The samples

become firmer, shorter and stiffer. Gain of calcium in the tissue particularly explained the mechanical changes but concentration and structural profile which developed in the tissue also promoted to the mechanical pattern (Torres *et al*., 2006).

The reduction of weight (WL %), loss of water (WR %) and solute (sugar) gain (SG %) were observed in osmotic dehydration of mango slices. The phenomena of mass transfer were affected by temperature and process time. Temperature and process time were different from the range of 40 to 120 minutes and 30 to 50°C respectively (Gabriela *et al.,* 2004). Osmotic dehydration process was done to increase the final quality of product. This pretreatment was done on banana and tomato rings, which helped to study of kinetics of osmotic dehydration, color properties and organoleptic evaluations. The results showed that much reduction of weight when 100% sucrose used as osmotic agent in banana. The tomato showed the highest values when 30% NaCl and sucrose: salt (1: 1.5) were used. The osmotic dehydration of tomato showed the lower chroma (C*) and redness values (a*) during osmotic dehydration (Ali *et al*., 2010).

Mechanism of osmotic dehydration

Osmotic treatment was done on the basis of minimum dehydration for food. The base of osmotic treatment was osmosis, physical phenomena motivated by variation in solute concentration of two regions which separated or divided by semi-permeable membrane, causing the water movement from low solute to higher solute concentration region with the help of membrane. When water consists of cellular tissue was wrapped in solution of hypertonic which low in molecular substances such as salts and sugars. The movement of solutes from solution to material and it dependent on difference of concentration between food material and solution which gave up two simultaneous counter flows and water outflow from material to solution (Shi and Le Maguer, 2003).

It dependent on the nature of nonselective cell membrane, the own soluble constitutes of product such as sugars, organic acids and minerals also traveled to the product along with outward stream of water. That's why this movement may be quantitatively unimportant to major types of mass transfer; it may be much resemblance with nutritional and sensory attributes of final quality of the product (Raoult-Wack, 1994; Azoubel and Murr, 2002; Sunjka and Raghavan, 2004). Transfer of mass continued till equilibrium osmotic dehydration was achieved. It was recommended that through capillary flow and diffusion, removal of water took place whereas uptake of solute to the product and leaching of the soluble solids of the product were only took place through diffusion (Shi *et al.,* 2009).

During osmotic treatment, food particles consisted of two phase behaviors in term of water and transfer of solutes. The dewatering of food material was well known to take place in high rate require more than few hours. After first several hours the rate of water loss slowly decreased in succeeding hours (6 hours) and finally flattens out. On the other way, solute impregnation into material was insignificant at the start of osmotic treatment, when dewatering rate was become lower then increased the solute rate into the material (Raoult-Wack, 1994). Early work on the osmotic treatment of food material was reported by Ponting *et al.* (1966), who explained the process as a moderate, non-thermal means of dehydration to generate good quality dehydrated fruit while decreased the original weight of the fruit to 50 % and preserved flavor and color.

On the basis of their pioneering work, osmotic treatment has attracted much attraction as practical processing method for fruits and vegetables. Although osmotic treatment has not much popular in the food of animal origin such as fish and meat. It should be clarified that osmotic behaviors of plant and animal were entirely different in terms of compositions and structures. This review was based only for the osmotic treatment of fruits and vegetables. Collignan *et al.* (2001) provided the review of literature on osmotic treatment of meat and fish products.

Osmotic treatment has many advantages over conventional methods; much of them include its mechanical simplicity, processed flexibility, and decreased the cost of energy because without any change, water can be removed. This process was done at room temperature to avoid the degradation of color, texture and nutritional values of the food. In this process loss of volatile compounds and oxidative changes was lowered (Raoult-Wack, 1994; Marani *et al.,* 2007).

Osmotic dehydration and infusion

There were two major divisions of osmotic treatment of foods such as infusion and osmotic dehydration. Although these two terms can be used interchangeably (Shi *et al*., 2009) and distinguished in scientific literature (Kuntz, 1995), the application and end-product properties were much different. The main reason of OD was to get maximum water removal from the product while lowering the solute uptake from adjoining osmotic solution.

On the other way, aim of infusion was to get the maximum transfer of external solutes into food with reasonable removal of water and maximum quality of final product (Raoult-Wack, 1994; Kuntz, 1995; Zhao and Xie, 2004). The process of infusion may also be called candying due to higher level of solute impregnation (Raoult-Wack, 1994). Osmotic dehydration completed within the day, whereas infusion took several weeks to complete (Zhao and Xie 2004). The reason was that water removal quickly took place at the start of osmotic process and latter on slowed down, while rate of solute gain increased.

The review of literature explained that most of work has been directed towards OD, and little bit research has been done to find out the ways to increase the solute gain and infusion efficiency. The reason was

that, methods which were used to prepare good the quality of infused or candied fruits being protected by patents (Mochizuki *et al.,* 1971; Kahn and Eapen, 1982; Tucker, 1997). This was most probably because the infusion was a beneficial process in which fruits can be impregnated with reasonably priced solutes (e.g., sugars) to get the substantial increase in product yield and weight (MacGregor, 2005).

In distinction, the literature provided the enormous amount of information on osmotic dehydration. It was usually recognized that osmotic dehydration did not effect to get the microbial stability (Azoubel and Murr, 2002). In addition, Marani *et al.* (2007) explained that osmotic dehydration could be an effective dewatering step to significantly reduce the energy requirement for freezing of fruits. The main advantage of solid uptake into material which occurred during OD, the valuable compounds and additives can be integrated in order to get better original nutritional and by taking and organoleptic properties of the raw material (Raoult-Wack, 1994; Torreggiani and Bertolo, 2001).

The osmotic dehydration has gained much attention in related to research, while dewatering impregnation soaking has coined to better explain the nature of process (Raoult-Wack, 1994; Torreggiani and Bertolo, 2001). Much research was conducted to investigate solid gain, kinetics of dewatering and developed mathematical model in order to characterize the osmotic behavior of food material. Such model has proposed for carrots tomatoes (Azoubel and Murr, 2004) and mango (Giraldo *et al*., 2003). These planned models were helpful in predicting transfer of mass phenomena and the control of various intrinsic and extrinsic factors of the process. However, the application of these models was limited due to variation of plants materials and structural responded of material to osmotic solution (Chiralt and Talens, 2005).

Process parameters of osmotic dehydration

Transfer of mass during osmotic dehydration inclined by temperature, size and geometry, concentration of osmotic solution, material to solution ratio, agitation, degree of solution, and methods of pre-drying. Temperature was much important factor which involved in breaking the integrity of plant material and membrane; for example plasma membrane started to undergo irreversible damage at 50°C (Thebud and Santarius, 1982). With increasing the level of agitation then was increasing the rate of dehydration. The sufficient level of agitation ensured the minimization of mass transfer affected on liquid side (Rastogi *et al.,* 2002). When the time spent over, then membrane did not provide barrier for the solute, which penetrated to the cell (Mauro *et al*., 2002).

The product mass ratio and solution was brought on different effects in the solution of dehydrated process. The driving force decreased to release of water when osmotic solutions become dilute. The shape of material was another factor in OD. If the size of solid material was bigger then dehydrate rate would be slowed because the length of diffusion path was higher. So process dependent on the food nature, structure and weight of osmotic solute and pressure was also affected on transfer of mass (Rastogi *et al.,* 2002).

Different types of osmotic solutions

The selection of solutions for osmotic treatment of food was of major importance; it provided driving force for concurrent flows of water and solute, then measured the rate and extent of solute uptake and removal of water as well as sensory and physical properties of the end product. It always careful to select the osmotic solutions to get the desire rate for processing and properties of the ending products. The ability of solutes in relations with other components of food was an important criterion for selection (Pan *et al.,* 2003).

The selection of cost for osmotic solution was very important. Although any solute which was dissolved in water can be used, the compounds that were commonly used as osmotic agent including sugars and sodium chloride (Raoult-Wack, 1994). Sugars were used for the osmotic treatment of fruits and sodium chloride had reported osmotic agent for vegetables (Contreras and Smyrl, 1981; Azoubel and Murr, 2004).

But when NaCl was used, the taste of product becomes salty that was not desirable (Lerici *et al.,* 1985; Azoubel and Murr, 2004). So sugar has been reported as excellent osmotic agent that provided many benefits that were inhibitors of polyphenoxidase, oxidative browning caused by enzymes in many vegetables and fruits. Sugar had beneficial in the respect of to protect the essential volatile compounds, which was helpful to restore the sensory properties of original food material (Ponting, 1973).

Sugars were further helpful to contribute stability of pigments and excellent retention of volatile compounds during drying of osmotically treated materials (Ferrando and Spiess, 2001). A combination of solutes was used to check the properties of materials. It had been reported that adding the small quantity of sodium chloride to the solution of sugar boosted up the osmotic drying force due to its lower molecular weight and higher capacity of decreasing the water activity (Lerici *et al.,* 1985; Taiwo *et al.,* 2003; Azoubel and Murr, 2004).

Kaymak *et al.* (1996a; 1996b) evaluated that the osmotic treatment of green peas with a sucrose/trisodium citrate solution after air drying (65°C, 10 % RH) improved the drying rate and rehydration quality of final product. They concluded that trisodium citrate helped in diffusion of water. The samples treated with sucrose or trisodium citrate retained the original color with more suitable flavor and texture when compared to non treated samples and those treated with sucrose (Kaymak *et al*., 1996b).

Molecular weight was another important factor that determined the rate and mass transfer, if

molecular weight of solutes was small (monosaccharides) then it penetrated into food more rapidly than higher molecular weight. The smaller molecular weight was desirable for the process of infusion then end product quality was excellent. On the other way solutes of higher molecular weight were selected carefully for osmotic dehydration to ensure the higher rate of water removal with little uptake of solute (Saurel *et al.*,1994; Kuntz, 1995). Among different types of solutions, sucrose was ideal as osmotic agents for OD of fruits.

It was recommended that by using of sucrose for OD of mango cut into slices which were helpful for maximum removal of water and gaining of solid uptake (Rincon and William, 2010). Sunjka and Raghavan (2004) reported high fructose corn syrup (HFCS) over sucrose for OD of cranberries as it produced maximum water loss and solid gain as compared to sucrose.

Factors related to Product

On the side of product, maturity, species and variety effected on structure of cell membrane, natural structure of tissues, soluble to protopectin ratio, insoluble amounts and entrapped air (Lazarides, 2001). The chemical composition (fat, protein, salt and carbohydrate), physical structure (fiber orientation, porosity and skin), may be effected by the kinetics of osmosis in food (Rahman, 2007).

Mainly porosity of the material was affected on transfer of mass rate and phenomena of shrinkage (Mavroudis *et al*., 1998a) as well as ratio of rehydration. The size and shape of the produce was affected by the surface area to volume ratio of the material with solution. So solute impregnation was controlled the surface phenomena, solute uptake was favored by high specific surface values (Lerici *et al*., 1985; Torreggiani, 1993).

Osmotic environment related factors

Environmental conditions played an important role during OD process of removing the water and migration of solutes. Environmental factors which influenced of solute gain and kinetics of the loss of water such as duration of time treatment and temperature. Less effect of temperature on solid gain but with the increased of temperature, water loss also increased (Beristain, 1990; Li and Ramaswamy, 2006a).

During OD of potatoes, by increasing of temperature up to 45°C, then ultimately increase the water loss and solid gain rates, in good deed of high water loss/solid gain ratios (Lazarides, 2001). Increasing the time of osmotic treatment, the mass transfer rates also increased until both solute and water concentration arrived at symmetry levels. OD for short period of time lowered the color loss during air drying of blueberries (Nsonzi and Ramaswamy, 1998).

However OD for longer period of time with sugar solution gave much loss of moisture and high solid gain. OD was improved by agitation around the syrup sample (Lenart and Flink, 1984; Mavroudis *et al*., 1998b). Generally concentration of osmotic solution, time treatment duration, temperature and level of vacuum were the major factors of osmotic process (Corrêa *et al*., 2010).

Benefits of osmotic dehydration

There were two important parameters of OD in food industry (1) quality feature of texture, color, flavor, stability of product, nutrients retention during storage and (2) energy competence. OD was discussed in respect of quality in many articles and also discussed in energy point of view

Quality issues

The concentration of OD was an important tool to reduce the water content with little bit damage on the quality of fresh products. This was done with the mild treatment of product at low temperature (30-50°C); so that temperature did not affect the properties of cell membranes, which was necessary to maintain the osmotic phenomenon (Lazarids, 2001). The plant tissue was continuously immersed in the osmotic medium because oxygen was not exposed so that there was no need of use of antioxidant to protect against enzymatic and oxidative discoloration (Dixon *et al*., 1976).

The immersion of food in osmotic medium before air drying was helpful for improving the final product quality since acidity of fruit reduced and prevents the oxidative browning (Ponting, 1973). Osmotic treatments before freezing were done to generate different types of fruits that stored for longer periods with the improvement of texture, flavor, and color after thawing (Sormani *et al*., 1999) and reduced the drip loss on freeze (Lazarides and Mavroudis, 1995).

Energy saving

Different types of OD applications were using in the processing of fruits and vegetables. However OD was not able to produce the product of low moisture content which has longer shelf life and stability. So osmotic dehydration was using with other drying methods such as freeze, vacuum or convective drying step to get the stable product. So that OD and drying methods were used in combination to reduce the cost of production. Water was removed in liquid form without using external energy (Lazarids, 2001).

Implementation problems of osmotic dehydration

Osmotic treatments for plants or animals material in concentrated solutions determined the factor due to executive of the concentrated salt or sugar solutions. Main problem occurred in managing the dilution rate. Food or solution ratio was controlled by the constant rate of the exchange of water or solution (Dalla Rosa and Giroux, 2001). Different technologies have been involved to control food or solution ratios (Dalla Rosa *et al.*, 1992).

The basic purpose of these technologies involved the spraying of solution on the food material, and then treated solution was collected and reused. Another problem associated to the implementation of osmotic treatment in the industry was the solute loss and particles from food such as acids, proteins, pigments and aromas which leached into solution. Major problem occurred in modification of pH, water activity as well as physical (viscosity) and sensorial (color and flavor) changes during utilization (Dalla Rosa and Giroux, 2001).

When solution was reused, then re-establishment of solute can be controlled. Several techniques had been used to get the objective, including evaporation at high temperature or low temperature under the application of vacuum, addition of solute to save the cost of energy, concentration of membrane and cryoconcentration. Microbial contamination by yeasts, molds, and lactic bacteria was most common during fruit and vegetable processing. Implementation of HACCP and Individualization of critical control points (CCP) methodology for control process become required when the osmotic treatment process was done without any succeeding process set up to get the stability of final product (Singh and Oliveira, 1994; Leistner and Gorris, 1995).

The product market of osmotic dehydration

The objective of osmotic dehydration was depending on the degree of stability. OD products that removed about 30 to 70% of water were ready to use and can be consumed as shakes or snake commodity. Osmodehydrated products can be utilized in bakery, dairy and candy industries. If food looked like fresh then 20 to 30% water can be removed by the process of osmotic dehydration. This process made the food to semi dried, frozen or treated with chemicals. This osmotic dehydrated food was utilized to produce the concentrates of vegetables and fruits. In France, Italy and Europe are the countries that have been used the modern methods for osmotic dehydration but in Asia, the OD of tropical fruits is become famous preservation method of fruits.

Robles-Manzanares *et al.* (2004) explained the dehydration and drying conditions to get quince (*Cydonia oblonga* Mill.) to be used as an ingredient in breakfast cereals. Pieces of Quince were dehydrated in the solution of fructose as concentration 45, 55 and 60ºBrix at 30, 40 and 50ºC. 45 and 55ºBrix at 30ºC, the high quality effect which were noted on color, vitamin C, water activity, ascorbic acid preservation and texture. García-Martínez (2002) prepared orange and kiwi jam from OD-treated fruits and to get products of high quality than commercially accessible.

Influence of temperature and concentration of osmotic solution

During osmotic treatment, when temperature increased then loss of water and uptake of solid took place (Saurel *et al.,* 1994; Ispir and Toğrul, 2009). In the literature of osmotic treatment, temperature around 50°C had been used for vegetables and fruits due to the subsequent reasons: 1) this reasonable temperature confined the deterioration of flavor, texture, and thermosensible compounds of the materials, 2) enzymatic browning and flavor deterioration of fruits start at temperature of 49°C (Ponting *et al.,* 1966), and 3) this temperature was also efficient to maintain the viscosity of the solution and adequate infusion time without changing the fruit quality.

It was reported that undesirable changes appeared on the blue berries at temperature of more than 50ºC (Shi *et al.,* 2009). Rahman and Lamb (1990) reported that temperature above 50ºC may not have a positive effect on solute gain during osmotic dehydration of pineapple with a sucrose solution (sample: solution (w/w) = 1:10). They concluded that sucrose were not capable to distribute as simply as water through the cell membrane at high temperature.

It was also reported that positive manipulate of high temperature on solute gain during the mixture of blueberries (sample: solution (w/w) = 1:1). When solution concentration increased it produced a positive effect on the rate of loss of water due to increase of the osmotic gradient. This has constantly reported for vegetables and fruits, when blueberries infused with different types of sugars (Shi *et al*., 2009).

The solute gain was assembled with high solution concentration had been reported (Ispir and Toğrul, 2009). This has been recognized to the increase of thick solute layer in the region of the product surface, which slowed the removal of water and created a situation which was more desirable for solute uptake (Nsonzi and Ramaswamy, 1998a). When solution of high concentration was used it had adversed effect on physical properties of the product.

Freezing of osmotic dehydrated products

Freezing of fruits was much important in modern society. The frozen fruits could be carried to that market where access of fruits could not be possible (Skrede, 1996). The freezing of fruits consequences in the better effect with relation to shelf life and availability of all over the year, so that different types of changes occurred during the process of freezing (Martinez-Monzo *et al*., 2002).

As preservation technique for fruits and vegetables, freezing is used to lower the temperature and water activity which linked to cryoconcentration for fruit liquid state during formation of ice crystals. Strawberries contained high freezable water content, so freezing implied to cellular damage and decreasing the product quality (Martínez-Navarrete *et al.,* 2001). The reduction of water content by dehydration treatments prior to freezing has been reported as a device for fruit cryopreservation, mainly reducing the freezable water content (Robbers *et al.,* 1997; Chiralt *et al.,* 2001, Martínez-Navarrete *et al.,* 2001).

Freezing is a method in which temperature of food lower down its freezing point. This method is used

to preserve food such as meat, vegetables and fruits. When water was freeze to ice then it prevent the enzymatic, chemical and microbiological activities (Ramaswamy and Marcotte, 2006). So this preservation method may be resulted in the change of quality such textural damage due to formation of ice crystals particularly in fruits case which contained high moisture content (Hung and Thomson, 1989). Water content of fresh fruits during OD process reduced the amount of water which was accessible to freeze (Li and Sun, 2002) and the result was that to decrease the change in the quality of frozen fruits (Tregunno and Goff, 1996). In addition, OD reduced the energy required for the formation of ice crystals, distribution and cost of packaging (Lowithun and Charoenrein, 2009). That's why OD was applied to upgrade the quality of frozen fruits (Li and Sun, 2002).

Freezing cause damage the cell structure during the formation of ice crystal. When aqueous solution was freeze, then remaining part of unfrozen water was acted as solvent for all solutes. This concentration determined the function of cell. High concentration of electrolytes influenced the ionic interactions, which may help to stabilize the state of proteins. Irreversible reactions took place when protein was denatured and unfolded. Further water and ice interacted with hydrophobic surfaces (Wolfe and Bryant, 2001).

Mechanical damage occurred from ice crystals when flexible components of cell were frazzled in areas where ice was present. During frozen storage, crystals went to metamorphic changes because systems move towards the state of equilibrium where free energy was minimized. Size of ice crystals carried on growing and applied extra stresses to the cell membrane (Kobs, 1997).

Repaid freezing was done for the commercial freezing of foods. So that's why cause of damage took to different forms such as toxicity was caused by effecting of concentration (Hebert *et al.,* 1981) or structural damages within cytoplasm. Cytoplasmic organelles were susceptible to the loss of functionality due irreversible endocytic vesicles (Dowgert and Steponkus, 1984) or lamellar to hexagonal transitions (Wolfe and Bryant, 1992) aggregation of membrane was due to a decline in charge density, and a lessening in free sterol content of membrane lipids (Uemura and Yoshida, 1986; Tregunno and Goff 1996).

Freezing caused rigorous changes in the properties of product and caused a remarkable texture loss due to cryoconcentration phenomena, which supported the denaturation of membrane. Osmotic dehydration as a pre-freezing treatment had been reported to lower the unwanted changes, which helpful in improving the quality of fruits (Conway, 1983; Forni *et al.,* 1990). If foods stored which was not in frozen stage then would continue to deteriorate.

The effect of osmotic treatment on the mango slices followed by freezing and stored at temperature of -18°C during 20 weeks was evaluated. Osmotic treatments lowered down the moisture contents, titratable acidity, vitamin C levels and lightness, while improving the total soluble solids. The samples treated with high concentrations of sucrose showed less change in properties during frozen storage. The less ripe fruit also has showed the lower acid while picking up sugar and has high vitamin C levels than that of mature fruit (Rincon and William, 2010).

In freezing of foods, formation of small and equally distributed ice crystal was much predicted (Khadatkar *et al.,* 2004). According to Sahari *et al.* (2004), these changes shoud be predisposed by period of storage, reaction of enzymes and microbiological changes. Urbany and Horti (1992) reported that method of freezing affected on pH. A similar result reported by Sahari *et al.* (2004) that have completed slow freezing of strawberries at -12°C, pH of the fruits was mentioned during storage and highest value of more than 3.4 that induced the anthocyanin damage in the fruits. The texture resemblance of osmodehydrofrozen kiwi fruit was much low and have great similarities for untreated and treated samples, but the texture of frozen apple treated with sucrose solution, high sugar and glucose contents were also increased (Marani *et al*., 2007).

The decrease of total soluble solids (TSS) in the mango slices after storing of one month. The nutrient loss of frozen mango slices during the freezing and storing of fruits were reported by Broto *et al.* (2002). During frozen storage of the tomato puree at temperatures ranges from -7°C to -18°C, the activity of lipoxygenase enzyme was still continuing, but it was decreased during four month storage (Calligaris *et al.,* 2002). Lisiewka and Kmiecik (2000) also evaluted that the activities of peroxidase, lipase, and catalase enzymes were still continuing in the frozen slices of tomato fruit stored at -20°C and -30°C. Freezing of food can be improved if food is previously osmodehydrated (Li and Sun, 2002).

A total of 93 % of dehydrofrozen apples were received by a sensory analysis panel in a study conducted by Bunger *et al.* (2004). Although, all records about the positive effect of OD on frozen materials, Talens *et al.* (2003) explained the volatile profile of kiwi fruit, OD pre-treatment did not cause a prominent change, if compared with fresh-frozen kiwi. Chiralt *et al.* (2001) analyzed the mechanical reaction of kiwi, mango and strawberry that has or has not been pretreated by osmotic dehydration. The cryoprotectant effect of the OD treatment was to get for strawberries that become firmer and tougher after freezing.

Dermesonlouoglou *et al.* (2005) indicated that osmotic treatment was a suitable method for the pre-treatment of freezing of watermelon. The effect of osmotic dehydration on cryoprotectant was noted to increase the color, texture, lycopene content and hardness compared with non-treated samples. When sensory analysis was done, the sample treated with high DE maltodextrin and oligofructose received better

acceptance than un-treated samples, both were stored for 180 days. Then it has suggested that osmotic dehydration was excellent method for freezing of fruits with brittle texture such as watermelon.

Freezing was best method to prevent damage of mango slices and thus had longer shelf stability. Mango slices immersed in the liquid nitrogen and checked the frozen properties. The study conducted that dipping the mango slices in liquid nitrogen for 0, 30, 40 and 50 seconds and four levels of storage periods for 0, 1, 2, and 3 months. The result showed that 40 seconds immersion of mango slices gave the better result for storage of 3 months, with pH of 4.9, TSS 14.07°Brix, total acid 0.46%, yellow color with brightness of 56.62, hue 85.09, chroma 39.57 and vitamin C content 27.66 mg/100 g, the product was preferred by the sensory penalists (Mulyawanti *et al*., 2010).

Some fruits and vegetables were not suitable for conventional method of freezing, due to degradation of texture, changing in color, nutritional losses and cost of energy. If pretreatment of OD was done to improve the poor quality of frozen tissues. The application of osmotic treatment of fresh fruits with substitute of osmotic solutes such as oligofructose and high-DE maltodextrin, at moderate temperature (35°C) caused significant amount of water loss and solid uptake. The non-treated samples suffered from degradation of texture and taste deterioration as compared with treated samples (Dermesonlouoglou and Taoukis, 2006).

Fruits texture was to be damaged by freezing. The reason was that fruits consist of much quantity of water, so ice crystals damaged the cellular structure of fruits. A decrease in moisture content was directly related to the water which available to freezing (Li and Sun, 2002), and if less quantity of water was frozen then it would less damage to fruits (Lazar, 1968). Therefore, a pretreatment was done to lower the water content and help in improving the quality of frozen fruits. Osmotic treatments for agriculture materials in 50% sucrose solution showed drip losses and tissues damage of osmodehydrofrozen products were much lower than that of non-treated samples (Ohnishi and Miyawaki, 2005). Osmotic treatment with sucrose syrup lowered the drip loss and moisture content of frozen pineapples.

Osmotic treatments with different levels of sugars affected the quality of frozen rambutan. The fruit pieces were dipped for 60 minutes before freezing at temperature of -40°C, compared with untreated samples. Then stored at -18°C to check the physical and chemical properties for 3, 60 and 120 days. The treated samples were good in taste, texture and acceptability (Lowithun and Sanguansri, 2009).

Texture quality of fruits and vegetables were improved during dehydration pre- freezing treatment (Huxsoll, 1982) and drip loss and structural collapse was reduced during thawing (Forni *et al.,* 1997). The fruit and vegetable products which are rich in vitamins, dietary fiber and minerals can be improved (Fito *et al*., 2001).

Freezing gave poor results, especially when frozen tomatoes were used for direct consumpti reported that quality of frozen tomatoes was poor when direct consumed. During freezing process, aqueous portion freeze out and formed ice crystals that breakdown uprightness of the cellular components. The osmotic status loosed the cellular membrane and permeability. The metabolic system was damaged, enzyme system was disrupted and turgor was loosed by cell.

The quality changes related to freezing may be led to break down of texture due to formation of ice crystals. Enzymatic activity changes the color which was induced by freezing process (Pinnavaia *et al.,* 1988). Before freezing, dehydration was applied which removed water from the product in such a way the amount of crystals decreased during freezing. OD was reported as pretreatment for freezing (Giangiacomo *et al.,* 1994; Giannakourou and Taoukis, 2003; Pinnavaia *et al*., 1988).

Urbanyi and Horti (1989); Biacs and Wissgott (1997); Calligaris *et al*. (2002) observed that progressive loss of color which may be took place in frozen tomato during storage. The similar results obtained by osmodehydrofrozen tomato samples. Color loss in untreated samples was more than treated samples. Initial loss of 56% ascorbic acid was observed in untreated samples evaluated that loss of 71% initial value of vitamin C was observed after 12-months storage at -20°C. Fuchigami *et al.* (1995) reported that decreased the pectin compounds were observed in the worsened texture of frozen carrots.

Osmodehydrofreezing

The combined process of OD and freezing is called osmodehydrofreezing which is used to get better texture properties of fruits and vegetables as well as lessen the structural collapse and drip loss. Giannakourou and Taoukis (2003) studied that change in quality of osmodehydrofrozen of green peas treated with maltitol and trehalose combined with $CaCl_2$ and NaCl and they observed that osmotic treatment lowered the quality changes in term of texture, color and retention of ascorbic acid for frozen samples.

The deprived quality of frozen cucumber can be increased by cryoprotection accomplished by pre-freezing step of OD. OD at mild heat treatment can protect the texture and develop flavor. The osmodehydrofrozen improved firmness during storage period. During sensory evaluation got excellent scores (Efimia *et al*., 2008).

Osmodehydrofreezing was combined process which improved the tomato quality during storage when compared the product by traditional freezing. Retention of ascorbic acid in tomato samples which was dehydrated with glucose and stored at temperature of -20°C for 1 year as compared to without treated

frozen samples (Dermesonlouoglou *et al*., 2007). The same results reported by Forni *et al.* (1997) in the pre-dehydrated and frozen apricots showed better retention of vitamin C than that of apricots freezing by the traditional methods. Moraga *et al.* (2006) checked the partial effect of dehydration before freezing of strawberries on the quality of final product. Much similar resulted reported by Maestrelli *et al.* (2001) in partial removal of water prior to freezing of melon. Osmodehydrofreezing has studied in several fruits: apricots (Forni *et al*., 1997), apples (Tregunno and Goff, 1996; Marani *et al.,* 2007; Blanda *et al.,* 2008), mangoes (Nunes *et al*., 1996; Chiralt *et al*., 2001).

Two processes involved in osmodehydrofreezing such as transfer of soluble solids and water between the material and osmotic solution and transfer of heat during freezing. Dehydration process was associated with transfer of heat process involved the freezing step. Apple was dehydrated with glucose and sucrose solutions and followed freezing was done in conventional air blast tunnel. A good result obtained by dehydration before freezing (Agnelli *et al*., 2005).

Dehydration and freezing

Frozen and dehydrated low moisture foods were in the form of amorphous metastable state, which was much sensitive to moisture and temperature. The conversion from liquid to the glassy state was the characteristic by discontinuous in physical properties such as dielectric constant, viscosity, free volume and coefficient of expansion which changed the other properties (White and Cakebread, 1996; Vanchy, 2002).

The chemical reaction occurred in frozen foods was the main determinant parameters which affected the quality of food after frozen storage. Oxidation of vitamin C, breakdown of lipids and protein precipitation were common example which decreased the quality of foods (Kerr *et al.,* 1993). The retention of vitamin C in frozen foods was known to be reliant on storage temperature and showed the presence of glass forming substances (Bork and Skibsted, 1997). The rate improvement in frozen cellular material was outcome of cell membranes being disrupted by change in pH, osmotic pressure and salt concentration, which allowed for enzyme-substrate interaction.

Osmo-freeze drying

There were two steps of freeze drying process (1) freezing the product and (2) dried the product by direct contact of sublimation of ice under low pressure. Freeze dried food after packaging that was stored for longer period of time, maintained the biological, physical, chemical and sensory attributes of the fresh material. In freezing process, pressure beneath the triplet point was subjected to frozen material. This technique was used for dried products, which hold high sensitive mechanism. However freezing was slow and luxurious method and commonly used for value added products (Cohen and Yang, 1995) and its application for vegetables and fruits were limited (Hammami and Rene, 1997). In accumulation to time consuming process needed extra energy to run the compressor and refrigeration units which were expensive for commercial use. The processing time required during condensation and freezing process was mainly dependent on water content and nature of fruits and vegetables.

Robbers *et al.* (1997) performed experiment on kiwi fruit to check the effect of osmotic dehydration during freezing. They carried experiment by dipping kiwi fruits in the sucrose solution (68% w/w) for 3 hours, after that they subjected fruits to air blast freezer with temperature of -30°C. They concluded that freezing started at low temperature in the dehydrated product and reduced the temperature of dehydrated products to -18°C in 20 minutes, which was 20 to 30% quicker as compared to untreated samples, so time required for untreated sample is 23-24 minutes.

Dehydrated foods with low moisture content always induced a low freezing point and less freezing time as far as there was less removed of heat and less water to freeze (Spiazzi *et al.,* 1998). They proved that reducing the moisture content may also be reduced refrigeration load during freezing which has important impact on the reducing of energy. Liu *et al* (2008) concluded that consumption of energy reached 35.7%, for condensed vapor 31.8% and 23.3% was used in vacuum pump of the total energy input. According to their statement about 67% of the total energy input was consumed in major drying process and strengthening of the vapor. Osmotic dehydration process reduced the moisture content has direct impact on freezing and condensation, the energy demand was reduced for whole freezing operation.

Osmotic dehydration and pre-freezing method

Dehydration pre-freeze treatments were helpful tool to lower the loss of cellular structure and chemical action of freezing on fruits (Huxsoll, 1982). Fractional water removal from the fruits before freezing led to depress the freezing and increased the microcrystallization and supercooling. The ratio of ice crystals was low in unfrozen state, with the subsequent reduction of sensory and structural properties. Literature indicated that fractional removal of water before freezing, referred to many fruits species (Torreggiani and Bertolo, 2001).

Industry used much energy to freeze the large quantity of water which present in fresh products. A subsequent reduction in moisture contents also reduced refrigeration load during freezing of fruits (Huxon, 1982; Torregianni, 1993). The main advantage was to concentrate the fruits before freezing also save the packaging and distribution costs and high quality products would be achieved due to reduction of structural collapse. It had confirmed that osmotic treatment improved the texture properties of thawed vegetables and fruits (Torregianni, 1995; Talens *et al.,* 2002) lowered the enzymatic browning and reduced

structural collapse and trickle loss during thawing (Forni *et al.,* 1990).

Conclusion

Osmotic dehydration (OD) is one of most important complementary treatment and food preservation technique in the processing of dehydrated foods, since it presents some benefits such as reducing the damage of heat to the flavor, color, inhibiting the browning of enzymes and decrease the energy costs. The main advantages of osmo-dehydrofreezing are not only economical but save energy, packaging and cost of distribution due to importance of product. The dehydrfreezing process also concerned with improving of quality.

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Effect of germination time on fat and protein contents, and α-amylase activity of Guinea Corn (*Sorghum vulgare*)

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Abstract

The effect of germination time on the malt quality of guinea corn was studied by germinating guinea corn for different period of time. Germination had a significant effect on α- amylase activity, fat and protein contents of guinea corn. The protein and fat contents of guinea corn decreased as germination time increases while the α- amylase activity increased as germination time increases. The fat content at day 1,3 5, and 7 was found to be 8.2%, 6.4%, 4.8% and 2.0% respectively. Protein content was also found to be 2.16%, 1.48%, 1.23% and 1.04% at the day 1, 3, 5 and 7 respectively while the α- amylase activity was found to be 1.88, 2.22, 3.22 and 4.44 µmole/min/ml at day 1, 3,5 and 7 respectively.

Key words: Germination, guinea corn, alpha-amylase, activity, malting, fat, protein

Introduction

Guinea corn is a cereal grain plant belonging to the family *Graminea* which has its origin from tropical africa. It is also cultivated in the United States, India, Palestine, Southern America and Southern Europe (Doherty *et al*., 1982). It is cultivated via kernel as a strong grass in a dry or moist soil which tolerate drought to a great extent, usually to a height of about 0.5 - 25.0 mm with leaves which are about 5.0 cm broad and 75.0 cm long. It has hermaphrodites flowers and it is pollinated by wind. It is a relatively fast growing crop which requires about 6 - 7 months slightly elevated temperature and moderate rainfall. In the United States, there has been an increasing tendency to give the minimum tillage with right conservation measures. Guinea corn is high in nitrogen and phosphorus which puts it in high demand and often its high energy level and posession of amino acids, although it is low in essential amino acids. Guinea corn has several uses which includes uses as food, feed for livestocks, and industrial raw materials. It stimulates respiration and improve digestion (Eggum *et al.,* 1983).

Malting process is very essential in brewing industries and soghum used in larger beer had more advantages than its disadvantages. Unmalted cereals like sorghum and maize are void of amylase enzymes which are synthesized during germination process. Malting promotes the synthesis of hydrolytic enzyme by the grains during germination (Banda, 1990). The pattern of biochemical events in early germination can thus be seen to be a direct expression of the operation of regulatory systems present in the grain at its resting period and as soon as grains begin to imbibe water there is spontaneous rise in respiration. The activation of seed during imbibition and germination may be a (CASCADE) process in which the imbibed seed contains only a limited amount of major enzymes, the product of reaction they (enzymes) catalyse induce other enzymes and the process continues until the metabolism of the seed is fully operational (Briggs, 1998). Metabolism of the specific mRNA can be investigated by studying the synthesis of the protein being investigated during germination.

During normal germination, protease activity developed and increased at 24 hours getting to its peak after about 3 days and thereafter shows a decline. Alpha- amylase is also greatly increased during germination, this evetually causes breakdown of the reserve starchy endosperm (Dhankner and Chauhan, 1987). Alpha amylase plays a vital roles in most plant tissues in the breakdown of starch and the only amylolytic enzyme that had any action in vivo on starch granules. On industrial scale amylases are the main enzymes required to break down starch to other sugars which is the first step in brewing process (Manner, 1974).

Alpha amylase (EC 3.2.1.1), hydrolyzes the internal α-1, 4 linkages in starch in a random fashion leading to the formation of soluble maltodextrins, maltose, and glucose. This enzyme is extensively used in starch liquefaction, brewing, food, paper, textile and pharmaceutical industries (Rasiah and Rehm, 2009; Rajagopalan and Krishnan, 2008; Gangadharan *et al.,* 2008; Thippeswamy *et al.,* 2006; Akpan *et al.,* 2004). Highly active enzyme is generally required for the conversion of starch into oligosaccharides. According to Aniche (1989) the activity of amylase increases with germination time reaching the maximum value on the sixth day of germination and declined on the seventh day of germination, he also reported that fat content decreased as germination time increased, the lower values were observed at the seventh day of germination (Aniche, 1989).

However, the present study is aim at looking at the effect of germination time on fat and protein contents as well as alpha amylase activity on the malting quality of guinea corn.

Materials and methods

Sample

Guinea corn (*Sorghum vulgare*) seeds was purchased at a local market near the Lagos State University campus Ojo, the sample were washed and soaked in water at room temperature for 24 h.

Germination

At the end of soaking, the water was drained off the grains and was allowed to germinate for seven days. At intervals some quantity of water was sprinkled on the germinating grains using spraying bottle. Sample of grains was collected at different days before spraying of water. The collected sample was dried in the oven for 6 - 7 h at 60 ^{o}C and then grounded with mortar and pestle for further analysis.

Fat content determination

This was done according to the AOAC (AOAC, 1984) methods. 5.0 g of the ground sample was weighed into a screw cap bottled, 20.0 ml of acetone was added and the tightly screwed bottle was shaken mechanically for 45 min. A sintered glass funned with a disc of glass filter paper was prepared, 10.0 ml of acetone was added, blown through air pressure and into a beaker. This was allowed to stand for 3 min. The content was put in the oven to enable the acetone to evaporate and later allowed to cool and weighed.

Protein content determination

This was carried out as reported by AOAC (AOAC, 1984). 250 mg of ground sample was weighed. 10.0 ml of Urea-SDS solvent was added and mixed. It was centrifuged at 3000 r.p.m for 45 min. The volume of supernantant was noted, 4.0 ml of Biuret reagent was added to 1.0 ml of supernatant and was allowed to stand for 10 min, the absorbance was taken at 540 nm.

Enzyme assay

This was carried out by determining total available carbohydrate using Clegg Anthrone method. 0.3 mg of the ground malted guinea corn was weighed, it was transferred into a 100 ml measuring cylinder, 10.0 ml of water was added and stirred with a glass rod to thoroughly disperse the sample. 13.0 ml of 52 % perchloric acid reagent was added. It was stirred frequently with a glassrod for 25 min. The content was made up to 100 ml and then transfered into 250 ml volumentric flask and made up to 250 ml. The flask was shaken and the content filtered into a test tube. 1.0 ml of filtrate was pipetted into test tubes, duplicate standard was prepared using 1.0 ml of diluted glucose standard solution, 5.0 ml of freshly prepared anthrone reagent was added to all the tubes. Tubes were mixed thoroughly and placed in a boiling water bath for 10 min, cooled quickly to room temperature by placing in a ice pocket. The optical densities of the sample and the standard were read at 620 nm against reagent blank.

Results and discussion

This study was designed to monitor the changes in fat and protein contents as well as alpha amylase activity in guinea corn during malting. FAO in 2003 (FAO, 2003) reported that guinea corn harvest contain relatively low amount of moisture and the starch is 3.4 %, sugar is 2.1 %, protein is 10 % and fat is 3.4 %. The protein content varies from species to species, because they are generally deficient in trytophan and lysine and most of the fats are found in the embrayo. The result of this study reveals that the fat content was 8.2 % on day one and the decrease was gradual until it reached 2.0 % on day seven (**Figure 1**). The increase in germination time also affected the values of protein content of guinea corn, the decrease was sharp as the germination day increases, from 2.16 % on day one to 1.04 % on day seven (**Figure 2**). This is probably due to hydrolytic action of enzymes which degrade endosperm storage protein into peptide and amino acids as germination proceed.

The germination processs significantly reduced the protein and fat contents of guinea corn but on the contrary alpha amylase activity was significantly increased as germination proceed. The alpha amylase activity was observed to increases with germination time. On day one alpha amylase activity was 1.88 µmole/min/ml, 2.22 µmole/min/ml for day 3, 3.22 µmole/min/ml for day 5 and 4.44 µmole/min/ml for day 7 respectively (**Table 1**).

Conclusion

This result reveals that as germination time increases protein and fat contents decrease with an increase in alpha amylase activity. Therefore, *Sorghum vulgare* could be an alternative to barley and wheat in beer fermentation.

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Figure 1: The percentage fat Composition in Malted Guinea Corn upon Days of germination

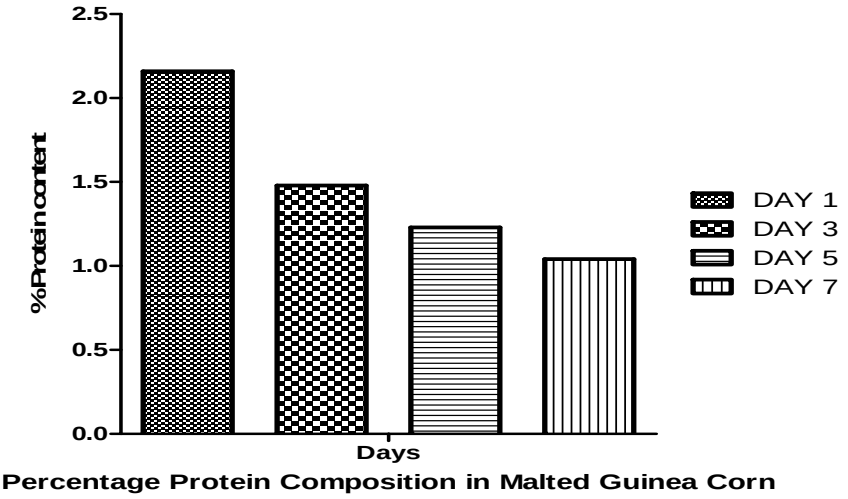

Figure 2: The percentage protein Composition in Malted Guinea Corn upon Days of germination

Table 1: The effect germination time on alpha amylase activity

DAYS	α- Amylase Activity (µmol/min/ml)
1	1.88
3	2.22
5	3.22
7	4.44

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Functional & technological aspects of resistant starch

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Abstract

Resistant starch (RS) has evoked a considerable position in human society due to its putative and positive impacts on health. Dietary starches are significant sources of energy for human being and contributions to health. Resistant starch plays important role in potential health benefits similar to soluble fiber and in functional properties. Resistant starch absolutely influences the function of the digestive tract, the blood cholesterol level and microbial flora. Resistant starch is also lower impact on the sensory properties of food compared with traditional aspects due to its swelling capacity, viscosity, gel formation and water-binding capacity which make it useful in a variety of foods. By this resistant starch (RS) has drawn considerable attention over the last two decades.

Keywords: Resistant starch, dietary fibre, prebiotic, glycaemic index, Glycaemic, physiological effects, gelatinization

Introduction

As the consumers request for superior quality of food products increases, the use of new technologies and ingredients increase. Numerous factors affected the consumer demand, including: health features like cholesterol, cancer, obesity, etc., changes in demographic features (ethnics, population ageing, etc.) (Pérez-Alvarez, 2008), the need for suitability, changes in supply systems and expenses. As a result of these changes, attention in new products, principally simple & easy to use products prepared using new technologies (Fuentes-Zaragoza *et al*., 2010), for example high pressures, etc., has intensely increased in recent years. The food industry offers quality and accessibility to a wide range of consumers including single households, working couples, the elderly population, and others (Pérez-Alvarez, 2008). To develop these types of products, one must assess consumer opinions, the most essential quality features being that they taste good, seems healthy and nutritive. Any functional food must be safe, healthy and tasty (Pérez-Alvarez (2008). The greater awareness in consumers of the relationship between a nutritious diet and well-being has been one of the reasons for the increase in popularity of novel food with good nutritional properties (Sanz *et al.,* 2008).

In the beginning of food science, it has been expected that the swallowed nutrients in the diet are not completely absorbed and used in the body. This will occur when these nutrients are swallowed in large amount else the term "accessibility" has come into share for their use (Southgate, 1989). The nutrients which are not digested primarily due to the indigestible cell walls, a huge structure, a low solubility, the presence of some compounds preventing the digestion, as well as components plentifully present in plant foods such as dietary fiber, phytic acid and tannic acid which completely lessen the digestion and absorption of some nutrients (Rosado *et al.,* 1987). There also arise a main problem is nutrient consumption in food processing because development of cross linkages in them, this makes the food unable for digestion and metabolism. Those parts of nutrients which are not available for digestion are known as "inaccessible" (Erbersdobler, 1989). Recently the problem of incomplete digestion and absorption of starch in the small intestine has arisen attention to get information about in non digestible starch elements (Cummings and Englyst 1991; Englyst *et al.,* 1992). These non digestible elements are called "resistant starches," and extensive studies and research have shown them that resistant starch have physiological functions similar to those of dietary fiber (Asp, 1994; Eerlingen and Delcour, 1995).

Resistant starch (RS) was considered complicating issue in the determination of total dietary fiber (TDF) levels by the Prosky Method (Englyst *et al.,* 1987). Dietary fiber takes its name from resistance starch mean "non-starch polysaccharides" (Asp *et al.,* 1988), it does not mean that starch, which interferes with the assay, should be the conventional fiber. Successive research on resistant starch has shown that it is not rapidly digested like normal starch. Resistant starch has been considered the fraction of starch which escapes from digestion in the small intestine and may be digested in the large intestine (Englyst, *et al.,* 1992).

Quantitatively starch is the major carbohydrate in the human diet which represent the primary source of energy that contributing to almost 60–70% of the total energy consumed and from whole of the consumed energy of which nearly 75% is obtained from of the starch granules of cereals and pulses (Asp, 1995; National Nutrition Monitoring Bureau, 1991).

Starch is considered the main foundation of carbohydrate in the diet of human (Ratnayake and Jackson, 2008). It is occur in the form of granules in many plant tissues with the range 1 and 100 lm in diameter but it totally depend upon the plant source. Chemically starches are polysaccharides composed of alpha-D-glucopyranosyl units that linked together with alpha-D- (1–4) and alpha-

D-(1–6) linkages. Alpha-D- (1–4) is the straight chain polyglucan found in amylose that comprised of approximately 1000 alpha-D-(1–4) linked glucoses; and amylopectin the branched glucan comprised of approximately 4000 glucose units with branches occurring as alpha-D-(1–6) linkages (Sharma *et al.,* 2008; Haralampu, 2000).

With the passage of time the greater awareness from the side of with respect to consumers to the nutritious diet and health has been one of the reasons for the increase in growth of fresh food with good nutritional properties (Pérez-Alvarez, 2008b; Sanz *et al.,* 2008a).

Dietary fibre (DF) which is considered the major part of the resistant starch can be defined (Buttriss and Stokes, 2008) that it is not an entity but a combined term which is the complex mixture of different substances with different chemical and physical properties. Dietary fibre is non digestible components of plants that are made up of the plant cell wall: cellulose, hemicellulose and lignin. The Commission of The European Communities (2008) gives the definition of 'fibre' as polymer of carbohydrate having three or more monomer units which are neither are digested nor to be absorbed in the small intestine.

In the past a lot of information has been written about starch structure, properties, biosynthesis and deprivation (Hoover, 2001; Tester and Karkalas, 2002). The level used to acquire information about resistant starch was obtained from the developing literature basis. Similarly molecular biology studies have made it possible to explore the complexity, the process of starch biosynthesis and a more complete view of the composition of the enzymatic practices involved.

The physico-chemical and structural uniqueness of starch vary on the basis of different botanical sources (Singh *et al.,* 2007; Singh *et al.,* 2009). A lot of research has indicated relationships between different starch characteristics and in vitro digestibility. Mostly the chemically modified starches are used in food applications to improve the functional properties than native starches, chemically modified starches show larger resistance towards a-amylase digestion (Han and BeMiller, 2007).

Resistant starches are those that are defined as the portion of starch which is not hydrolyzed by the enzymes in the small intestine and passes as sigh to the large intestine. Most important method widely used to classify the starches is based on kinetics (Englyst *et al.,* 1992).

Resistant starch and its classification:

Resistant starches (RS) which are starches that are not engrossed in the small intestine plays a vital role in human health or resistant starch is a type of carbohydrate formed by plants that performs like dietary fiber in the body. Distinction from other types of starch, resistant starch (RS) passes through the small intestine undigested and travels to the large intestine where it is fragmented down and fermented. Like dietary fiber, resistant starch plays a significant role in gastrointestinal function. **Resistant starch (RS1)** is that type of starch which is resistant to digestion because of physically unapproachable to digestive enzymes such as in seeds or legumes and natural whole grains. **Resistant starch (RS2)** is that type of resistant starch that occurs in its natural grainy form, such as uncooked potato, green banana flour and high amylose corn. **Resistant starch (RS3)** is a resistant starch that is formed when starchy foods cooked and cooled such as in bread, cornflakes and cooked-and-chilled potatoes or retrograded high amylose corn. **Resistant starch (RS4)** is that type of starches that have been chemically improved to resist digestion. This type of resistant starches can have a extensive range of structures and are not found in nature. Normally resistant starches are naturally exists in foods like legumes, unripe bananas, and cooked and cooled potatoes. Some treated foods such as breakfast cereals and breads also have additional resistant starch called Hi-Maize resulting from corn (Thompson, 2000). Resistance starch is starch that resist digestion in the small intestine of normal healthy persons and it is known as the third type of dietary fiber, as it can supply many benefits of insoluble fiber and soluble fiber.

Based on enzyme activity:

According to Berry (1986) when enzyme act on starches can be classified as follows.

Rapidly digestible starch (RDS): quantity of glucose discharge after 20 min. mostly found in amorphous and dispersed form in starchy foods like potato and bread.

Slowly digestible starch (SDS): quantity of glucose free between 20 and 120 min of in vitro digestion that is physically inaccessible starches found in cereals.

Resistant starch (RS): total starch minus quantity of glucose released within 120 min of in vitro digestion which can be explained by the following equation

RS = TS – (RDS – SDS)

Where TS is the total starch contents

Based on nutritional characteristics

According to (Nugent, 2005) starches are divided on nutritional characteristics are given below.

Digestible starches (DS)

Those starches which are digestible by body enzyme which include rapidly digestible starch (RDS) and slowly digestible starch (SDS).These starches are considered to be digested in small intestine but their digestion is slow than others.

Resistant starch (RS)

That starch which is not digested body enzymes which are further divided in RSI, RS2, RS3 and RS4.

RSI: These are physically protected which resistant milling and chewing.

RS2: These are ungelatinized starches which are slowly hydrolyzed by alpha amylase and they resist to food processing and cooking.

RS3: These starches are retrograded which resist to processing conditions.

RS4: These starches are chemically modified which have cross linking that show less susceptibility to digestion.

Why resistant starches are not digested

The tough outer coating makes it physically accessible to digestive enzymes like various amylases and explains the resistant nature of raw starch granules (Haralampu, 2000). And also resistant starches are physically inaccessible to the digestive enzymes as in grains, seeds and tubers.

(ii) The starch granules are structured in such a way that digestive enzymes are prevented from breaking them down. These types of starches are present in raw potatoes, unripe bananas and high amylase maize starch (Nugent, 2005).

(iii) The cooling of Starchy foods which have been heated in excess water leads to the formation of crystals which hinder the digestion process the whole phenomenon is known as gelatinization. Some times the cooking which is done in water leads to the preparation of starchy foods for consumption that render the digestion of food (Haralampu, 2000).

(iv) Sometimes chemical treatment makes selected starches resistant to breakdown by digestive enzymes due to cross-bonding formation (Lunn and Buttriss, 2007).

Digestion of starches

The morphological uniqueness of starches from different botanical sources varies with respect to genotype. The variation in the morphological characteristic like such size and shape of starch granules is credited to the biological origin (Singh *et al*., 2007). Initially (Langworthy and Deuel, 1922) suggested a visible clear negative relationship between large size granules and starch digestibility. After that various studies were taken under consideration to validate this relationship. The group of researcher (Lindeboom *et al*., 2004) predicted that the small barley and wheat starch granules are hydrolyze quicker than the large granules. After that (Kaur *et al.,* 2007) reported that significant differences was exist among the values obtained by enzymatic hydrolysis of native potato starches and their different small, medium and large fractions.

Food sources of resistant starch

Starch which is the most important carbohydrate and considered as the major dietary source , it is the abundantly found in the form of polysaccharide in plants and found as a granules in the chloroplast of green leaves and the amyloplast of seeds, pulses and tubers (Sajilata *et al*., 2006). Resistant starch is in nature found in all cereal grains, seeds and in starch-containing foods (Charalampopoulos *et al*., 2002).

Pulses are the dicotyledonous seed of plants which belong to the family Leguminosae that contain starch in abundantly in the pulse seed accounting for 22–45% of the seed itself. Wide spread research on cereal, potato, sweet potato and cassava starches have confirmed that they can be readily available for use in food and non-food applications. There is a lack of information on the molecular structure of pulse starches (Hoover and Ratnayake, 2002).

How resistant starch is formed

Starch is present in plant tissue in the form of granules which have diameter in range between 1 and 100 mm. Starch is made up of two molecules amylase the straight chain polyglucan and amylopectin the branched glucan (Zobel, 1988). These starch granules are tightly packed and they can be hydrated. This compact structure of starch make inaccessible to digestive enzymes. When Starch granules are heated in excess water that becomes disrupted and whole process is known as gelatinization that prevents the starch molecules fully accessible to digestive enzymes. After heating then cooling of starch is done then relatively slow re-association of starch molecules is occur this process is commonly termed retrogradation of starch (Colonna *et al.,* 1992) during this process starch molecules re-associate themselves and can form tightly packed structures which have strong hydrogen bonding. This structure is thermally very stable and can not easily be rehydrated (Jane and Robyt, 1984).

Beneficial physiological effects of RS

There are many physiological effects which have been ascribed to RS (Nugent, 2005) that proved to be beneficial for health. Resistant starches have got much attention for both its prospective health benefits and useful properties (Sajilata *et al*., 2006).

Improved glycaemic response

Resistant starch delay increases in blood glucose when have to take a meal by slowing the rate and amount of carbohydrate digestion and making it ideal for people with diabetes. This more controlled glycaemic response also helps to suppress hunger and maintain energy levels up all over the day. Those foods which containing resistant starches moderate the rate of digestion and this slow digestion of resistant starches has implications for its use in controlled glucose release applications. Digestion which occurs over a 5- to 7-h period reduces postprandial glycemia and insulinemia and has the potential for increasing the period of satiety (Reader *et al.,* 1997).

Prevention of colonic cancer

The research has shown that butyrate may reduce the threat of evil changes in cells. Keen studies in the cecum of rats fed resistant starch have shown that increase in fecal bulking and lower in fecal pH are linked with the decreased incidence of colon cancer (Tharanathan and Mahadevamma, 2003).

When resistant starches were combined with an insoluble dietary fiber like wheat bran much higher SCFA levels in particular butyrate was observed in the feces (Leu *et al.,* 2002).

Resistant starch as a prebiotic

Resistant starch has been recommended for use in probiotic compositions to encourage the growth potential of beneficial microorganisms such as Bifidobacterium

(Brown *et al.,* 1996). Since resistant starch is entirely passes the small intestine and that behave as a substrate for growth of the probiotic microorganisms.

Prebiotics are food ingredients which non-digestible and play beneficial affect on the host by stimulating the activity of one or more bacteria (probiotics) in the gastrointestinal tract and promoting the health beneficial effects (Scholz-Ahrens *et al*., 2007). The most distinctive form of prebiotics are inulin and oligofructose which both naturally present in a number of fruits and vegetables like bananas, chicory, Jerusalem artichokes, onions, garlic and leeks, and wheat (Buttriss and Stokes, 2008).

Resistant starch as a component of dietary fiber

Resistant starch proved to be highly resistant to digest by mammalian enzyme and it is considered as a component of fiber on the basis of the present definitions of dietary fiber. While some part of the resistant starch consist of low-molecular-weight dextrins, the bulk of polymers and retrograded amylase forms the major fraction (Ranhotra *et al.,* 1991a).

The resent study have proved that resistant starch behaves physiologically like fiber which is insoluble and exhibits a level of slow digestibility and can be used as a vehicle for the slow release of glucose. The resistant starch like soluble fiber also have positive impact on colonic health by increasing the crypt cell production rate and decreasing the colonic epithelial atrophy in comparison with no-fiber diets (Haralampu, 2000).

Resistant starch inhibits fat accumulation

Substitute of 5.4% of total dietary carbohydrates with resistant starch in a meal could considerably enhance postprandial lipid oxidation suggestive of reduction in fat accumulation in the long term (Higgins et. al 2004).

A group of researchers have examined the prospective of resistant starch to alter fat oxidation and various studies have predicted its potential as satiety agent. (Sharma *et al*., 2008).

Recent studies on humans indicate that diets rich in resistant starch do not affect total energy expenditure, carbohydrate oxidation and fat oxidation (Raben *et al*., 1997). In further study on human volunteer's breads rich in resistant starch impart greater satiety than white breads between 70 and 120 min after eating (De Roos *et al.,* 1995). Another group also reported that high resistant starch meals caused less satiety than low resistant starch meals one hour post ingestion (Anderson *et al.,* 2002)

Resistant starch reduces gall stone formation

The digestibility of starch present in rice and wheat is improved by the process milling which produce flour (Heaton 1988). The digestible starch contributes formation of gall stone through a greater secretion of insulin and as a result insulin leads to the stimulation of cholesterol synthesis, so resistant starches are found to reduce the occurrence of gallstones (Malhotra, 1968).

Gallstones are present in fewer amounts in the people southern India because where people consume whole grains rather than flour. The dietary intake of resistant starch is 2- to 4-times lower in the United States, Europe, and Australia when compared with the people of India and China which have less chances gallstone formations (Sajilata *et al*., 2006).

Resistant starches role in absorption of minerals

Resistant starch plays important role in the absorption of a number of minerals in the ileal of rats and humans (Lopez *et al.,* 2001) and a group of scientist (Younes *et al.,* 1995) also reported an better absorption minerals is done like calcium, magnesium, zinc, iron and copper in rats which feed by rich resistant starch diets but in humans these effects appear to be limited to calcium (Coudray *et al*., 1997).

Resistant starches as a weight control

Resistant starch also provides benefits to individuals who are trying to attain and sustain a healthy body weight. Due to this resistant starch are indigestible in nature and provides fewer kilojoules (calories) than digestible starch and should becomes a part of healthy balanced diet (CSIRO Division of Human Nutrition, 1996)

How can I increase my resistant starch intake?

This recommendation was given in (CSIRO Division of Human Nutrition, 1996).

- Addition of soaked legumes like red kidney beans and chick peas to your soups, casseroles and salads.
- Addition of butter beans in your mashed potato or pumpkin.
- Use whole grains, seeds and cereals in your diet like oats, barley corn and linseeds
- Eat more fruit like bananas before it is ripe.
- Eat more salads that have been cooked and cooled, such as potato salad, rice salad and pasta salad.
- Eat breads and cereals with added resistant starch.

Conclusions

Resistant starch offers a much amazing attention for both its potential health benefits and functional properties as a ingredients. It shows physiological effects just like soluble fiber. Resistant starch as a functional fiber makes feasible the formulation of a lot of food products with enhanced consumer adequacy and better sweetness than those products which made with traditional fibers. Resistant has changed the life style of people which promoted the reasonable reduction of fruits and vegetables use. Resistant starch has properties analogous to fiber and shows hopeful physiological benefits in humans which reduce occurrence of the disease. The foods which contain higher quantity of resistant starch has give up less calories and inferior glycaemic loads which is important for diabetics as well as the weight conscious peoples. Recently it is recognized source of fiber and classified as a fiber component with partial or complete fermentation. Exactly, it is feasible to increase

the contents of resistant starch in foods by changing the processing conditions like pH, time, temperature, number of heating and cooling cycles, freezing and drying.

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Quality evaluation of different brands of Tetra Pak mango juices available in market

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Abstract

The study was conducted to evaluate the quality of six different brands of mango juices packed in Tetra Pak for nutritional quality evaluation. Physicochemical characteristics, minerals, microbiological and sensory characteristics were determined for all the samples. Three samples were found to contain total soluble solids less than the standard limit prescribed by Pakistan Food Laws. Same samples also showed poor sensory attributes. Sample (V) showed the highest TSS, total sugar and vitamin C contents. No sample was found contaminated with *E coli* and *Salmonela*. Sample VI got highest scores for taste (8), flavor (8), texture (8) and overall acceptability (8).

Key words: mango, juice, acidity, sugars, ascorbic acid and minerals.

Introduction

Mango is king of fruits. Mango is one of the most popular and loved fruit. It is a tropical fruit which belongs to the genus *Magnifera indica* and family *Anacardiaceae*. Hundreds of varieties of mangoes are known to exist in worldwide. The main varieties of Pakistan are Dusehri, Chounsa, Anwar Ratul, Malda, Fajri, Saroli, Sindhreri, Langra, Desi, Almas and Totapari. These varieties are different in color, size, shape, flavor, and taste. In Pakistan, total area under mango cultivation is 84800 hectares, with annual production of 839300 tones. Punjab province contributes more than 50 % towards total production (Khan *et al*. 1995).

Mango is mostly consumed as fresh fruit, but due to its perishable nature it can not be stored for long time. In order to make the mango fruit available during off season, it is processed to make juices, jams, squashes, nectars, chutney, pickles, toffees and canned mango slices etc. Mango is one of the cherished fruit not only for taste but also for nutritional values. In India mangoes are used as a blood builder, because of their high iron contents. They are suggested for treatment of anemia and beneficial to women during pregnancy and menstruation. People who suffer from muscle cramps, stress and heart problems can benefit from high potassium and magnesium contents that also help those with acidosis. Mango fruit is also beneficial in the treatment of nephritis as well as other kidney troubles (Islam 1986). It serves as good source of energy, vitamins A, vitamin C, iron and phosphorus etc. (Malik 1994).

A large number of new brands of fruit juice based beverages have appeared in the market in glass & plastic containers and brick pack. Although, food laws exist for the production of quality food products (Awan 1985), yet most manufacturers do not strictly comply with these laws. Food adulteration can prove very dangerous for the development of a healthy society. It can lead to a number of diseases such as paralysis, cancer, mental retardation and hypertension etc. Therefore it is essential to take necessary steps to check food adulteration etc. Adulteration and contamination in edibles especially beverages, bottled water, cooking oil/ ghee, spices, tea, sweeteners like sugar, sweetmeats, bakery products, milk and milk products, fruit and vegetable products are constant threat to the health of common man. One of the important measures in this regard is to create awareness amongst the public regarding the hygienic conditions. The kinds of impurities found in food items sold in the markets should be highlighted. This can only be done by media through advertisements. Government should start campaign against food adulteration, forcing the producers to change their methods of production. Keeping in view this fact the present study was undertaken to evaluate quality and nutrition value of the different mango juices available in the market.

Materials and methods

Six commercially Tetra Pak (ready to drink) mango juices were collected from different local markets of Peshawar and labeled with laboratory codes. These samples were analyzed for microbial contamination, physico-chemical characteristics and sensory evaluation in Food Technology Centre, PCSIR Laboratories Complex, Peshawar, Pakistan.

Microbiological Analysis: Total Plate Count (TPC), fecal *coliform*, *E. coli*, *staphylococcus aureus* and

salmonella were determined according to the standard methods (APHA (2005). Agar was used as a cultural medium. *Coliform* bacteria were determined by the most probable number technique of serially diluted samples. The fecal *coliform* bacteria were found out by inoculation where as *Staphylococcus aureus* were estimated by using blood agar for the isolation of colonies. *Salmonella* were investigated by using lactose broth as pre-enrichment medium. The entire tests were carried out by the methods described in APHA (2005).

Physico-chemical Analysis: Moisture and crude protein were determined by the methods as reported in AOAC (2005). Acidity was determined by titrating diluted sample against 0.1 N NaOH according to AOAC (2005). The pH was recorded on pH meter (HANNA Model HI 8520). Total soluble solids were directly recorded by digital refractometer (Atago RX-1000) and the results expressed as percent soluble solids (Brix) as described in AOAC (2005). Sugars were determined by using Lane and Eynon's method as given by Ruck (1969). Ascorbic acid content of the samples was estimated by indophenol titrimetric method. Ash was determined by incinerating the samples at 525 ^{0}C to white ash (AOAC, 2005).

Mineral Analysis: Dried samples were first digested with nitric acid and perchloric acid and then the aliquots were used for the determination of minerals. Iron, manganese and calcium were determined by Perkin Elmer Atomic Absorption Spectrophotometer whereas sodium and potassium were estimated through Flame Photometer (AOAC, 2005).

Sensory Evaluation: Samples were evaluated by a panel of six judges for sensory characteristics like color, taste flavor, texture and overall acceptability at room temperature as described by the Larmond (1977). Scoring was done according to 9-Point-Hedonic Scale.

Result and discussion

This study was conducted to evaluate the quality of juices by studying their physio-chemical parameters, mineral analysis, microbiology and organoleptic characteristics.

Physiochemical characteristics: The results of the physico-chemical analysis are given in Table 1. It is evident that moisture content was found maximum in sample IV (94%) and minimum in sample V (83.5%). Samples I and III were found to have same moisture content (93%) followed by sample II (98%) and sample V (86%). High moisture content was very important factor affecting the flavor of mango juices. Product having high moisture content has minimum shelf stability Ayub (2005). Ash contents reveal cumulative pictures of different minerals present in the food. The ash content of given mango juices were ranged from 0.37% to 0.13%. Maximum ash contents were found in sample II (0.37%) and minimum in sample III (0.13%). The variations in ash contents of the samples may be attributed to the formulations of each manufacturer. The lower ash content indicates low fruit contents in the drinks. The variations in brands might be due to the raw material, recipe or the ingredients used. Length of storage and temperature also affect the quality parameters of these beverages (Hussain 1993).

Protein is the most important component of food. Result showed that mango juices were very poor source of protein. High level of protein content was investigated in sample VI (0.179%) and low level for sample IV (0.016%), whereas sample I and sample II were found in same range regarding protein contents (0.175%). Results were in agreement with the value reported by Akubor (1996). The values of pH were recorded in the range of 4.2 to 4.6. The higher pH was in sample IV followed by sample III and V (4.56). The higher pH values may be due to the loss of acidity. Sivakov (1990) also observed a rise in pH of fruit juices during storage. Acidity plays a very important role in the flavor of the products Ullah (2005). According to results acidity was higher in sample IV (0.259%) and lower in sample III (0.098%). Akubor (1996) has also analyzed acidity for mango juices and reported that acidity was increased with increasing period of storage. Sample V was found higher for TSS whereas sample I and IV were found lower for TSS. Akubor (1996) has found TSS up to 4.2 % in mango juices. Increase in TSS during storage has been reported by Mahajan (1994). Three samples were found to contain total soluble solids less than the standard limit prescribed by Pakistan Food Laws.

Pectin was found almost in the same range in all the given samples. Jones (1951) determined that degradation of pectin substance and formation of free uronic acid in guava resulted in an increase in acidity. Fermentation depends on sugar concentration in given samples. Total sugar was found in the range of 11.8 to 5.0%. Sample No. V contains the highest total sugar while sample No. I and IV contain the lowest total sugars. Akubor (1996) reported 3.6% total sugar in mango juices.

Ascorbic acid is an important constituent of fruits. Its deficiency produces a disease called scurvy. It is the most difficult of the vitamins to preserve during dehydration and blanching (Rauf 1988). The maximum level of ascorbic acid (3.63%) was found in sample V followed by sample IV (2.34%).

Mineral composition: Calcium, magnesium, sodium, potassium and iron concentration varied widely among different brands of Tetra Pak mango juices (Table 2). Calcium ranged from 24 to 70 ppm, the maximum being found in sample V & VI and the minimum in sample III. Magnesium ranged from 54 to 134, sodium 70 to 178, potassium 29 to 104, iron 0.4 to 5.5 ppm.

Microbiological analysis: Microbiological analysis of juices showed that *E. coli* and *Salmonella* were not found in any of the samples (Table 3). *Total coli form* and *Fecal coliform* were < 1.1 MPN in all the samples. Total Plate Count (TPC) varied from 0 to 20 MPN and *Staphylococcus aureus* 0 to 4 MPN in different samples of Tetra Pak mango juices.

Sensory evaluation: The variation regarding sensory characteristics in different brands of juices for color, taste

Table 1. Physico-chemical analysis of different brands of Tetra Pak mango juices.

Characteristics	Samples					
	I	II	III	IV	V	VI
Moisture %	93	88	93	94	86	83
Ash %	0.29	0.37	0.13	0.27	0.19	0.33
Crude Protein%	0.175	0.175	0.145	0.016	0.133	0.179
pH	4.21	4.26	4.56	4.4	4.56	4.6
Acidity %	0.189	0.102	0.098	0.203	0.231	0.259
TSS %	5.1	9.8	6.5	5.1	12.9	10.3
Pectin %	0.048	0.027	0.031	0.036	0.046	0.026
Total Sugars %	5.0	9.28	6.25	5.0	11.8	9.31
Vitamin C mg/100 g	2.72	3.14	2.56	2.34	3.63	3.04

Table 2. Mineral analysis of different brands of Tetra Pak mango juices.

Minerals	Samples					
	I	II	III	IV	V	VI
Calcium(ppm)	32	60	24	44	70	70
Magnisium (ppm)	134	54	78	88	54	96
Sodium (ppm)	79	178	130	86	70	143
Potassium(ppm)	69.5	29	45	49	104	55
Iron(ppm)	2.6	0.4	0.7	2.9	2.6	5.5

Table 3. Microbiological analysis of different brands of Tetra Pak mango juices.

Microbes	Samples					
	I	II	III	IV	V	VI
E. coli (MPN)	-ve	-ve	-ve	-ve	-ve	-ve
Salmonella (MPN)	-ve	-ve	-ve	-ve	-ve	-ve
Total coliform (MPN)	< 1.1	< 1.1	< 1.1	< 1.1	< 1.1	< 1.1
Fecal coliform (MPN)	< 1.1	< 1.1	< 1.1	< 1.1	< 1.1	< 1.1
Total Plate count (MPN)	10	1	20	14	0	0
Staphylococcus aureus (MPN)	3	0	1	4	0	0

Table 4. Sensory evaluation of different brands of Tetra Pak mango juices.

Samples	I	II	III	IV	V	VI
Color	6.0	7.0	8.0	5.0	7.0	7.0
Taste	5.0	7.0	7.0	5.0	8.0	8.0
Flavor	7.0	8.0	7.0	7.0	7.0	8.0
Texture	6.0	7.0	6.0	4.0	8.0	8.0
Overall acceptability	5.0	7.0	6.0	5.0	7.0	8.0

and flavor might be attributed to ingredients, recipes and processing. Organoleptic attributes decreased with increase in storage period Awan (1993). Marketing conditions also play a significant role in quality deterioration of drinks Siddique *et al* (1987). Ahmad *et al* (2011) conducted study to evaluate the quality of five different mango squashes available in Lahore market. The results showed that there were significant differences among squashes of all brands for physio-chemical parameters. However, all the mango squashes were acceptable regarding organoleptic quality. In the present study highest score for color was exhibited by sample III. However, sample II, V and VI showed same score (7) for color. Taste of samples V and VI was found better. These samples exhibited highest scores (8) for taste. The samples which exhibited less total soluble solids than the standard limit prescribed by Pakistan Food Laws also showed poor sensory attributes. Sample VI got highest scores for taste (8), flavor (8), texture (8) and overall acceptability (8).

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Cumin (*Cuminum cyminum*) as a potential source of antioxidants

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Abstract

Spices are the building blocks of flavor in food. Their primary functions are to provide aroma, texture and color to food. In addition they also act as preservative, and provide nutritional, and health benefits. Cumin (*Cuminum cyminum*) locally known as 'zeera' is a flowering plant in the family Apiaceae. It is commonly used as a condiment and flavoring in many eastern dishes. Cumin is known for its antioxidant properties. The most important chemical component of cumin fruits is essential oil content, ranging from 2.5% to 4.5% which is pale to colorless depending on age and regional variations. Studies of the chemical composition of cumin oil from different countries showed the presence of the following components: α-pinene (0.5%), Myrcene (0.3%), limonene (0.5%), 1-8-cineole (0.2%), p-menth-3-en-7-ol (0.7%), p-mentha-1, 3-dien-7-ol (5.6%), caryophyllene (0.8%), β-bisabolene (0.9%), β-pinene (13.0%), P-cymene (8.5%), β-phellandrene (0.3%), D-terpinene (29.5%), cuminic aldehyde (32.4%), cuminyl alcohol (2.8%), β-farnesene (1.1%) together with much smaller quantities of α-phellandrene, α-terpinene, cis and trans sabinene, Myrtenol, α-terpineol and phellandral. In addition to volatile oil cumin also contains nonvolatile chemical components including tannins, oleoresin, mucilage, gum, protein compounds and malates. The total phenolic content of methanolic extracts of different cumin varieties (cumin, black cumin and bitter cumin) ranged from 4.1 to 53.6 mg/g dry weight. In this comprehensive review focus will be on the antioxidant and flavoring compounds of cumin.

Keywords: Spices, cumin, Essential oils, antioxidants,

What are Spices?

Spices are non-leafy parts (e.g. bud, fruit, seed, bark, rhizome and bulb) of plants used as a flavoring or seasoning, although many can also be used as a herbal medicine. The term 'spice' originated form the Latin word 'species', meaning of specific kind. A closely related term, 'herb', is used to distinguish plant parts finding the same uses but derived from leafy or soft flowering parts. The two terms may be used for the same plants in which the fresh leaves are used as herbs, while other dried parts are used as spices, e.g. coriander, dill. Spices have many functions in food. Primarily they are used for flavoring the food products but in addition they are also used in preservation of food and provision of nutritional and health benefits (Nazeeem, 1995).

Spices have a profound influence on the course of human civilization. They permeate our lives from birth to death. In everyday life, spices succor us, cure us, relax us, and excite us. Ancient peoples such as the Egyptian, the Arab and the Roman made extensive uses of spices, not only to add flavor to foods and beverages, but as medicines, disinfectants, incenses, stimulants and even as aphrodisiac agents. In Europe, Middle East and Asia they were used to preserve meat, bread and vegetables. No wonder they were sought after in the same manner as gold and precious metals. There are many forms in which spices are available e.g. fresh, dried and frozen; whole, ground, crushed, pureed, as pastes, extracts, or infusions (Raghavan, 2007).

Spices are generally composed of fiber, carbohydrate, fat, sugar, protein, gum, ash, volatile (essential oils), and other nonvolatile components. All of these components impart each spice's particular flavor, color, nutritional, health, or preservative effects. The flavor components (volatile and nonvolatile) are protected within a matrix of carbohydrate, protein, fiber, and other cell components. When the spice is ground, cut, or crushed, this cell matrix breaks down and releases the volatile components (Raghavan, 2007).

Essential oils are the major flavoring constituents of a spice. They are soluble in alcohol or ether and are only slightly soluble in water. They provide more potent aromatic effects than the ground spices. Essential oils lose their aroma with age. Each essential oil has many chemical components, but the characterizing aroma generally constitutes anywhere from 60% to 80% of the total oil. Essential oils are very concentrated, about 75 to 100 times more concentrated than the fresh spice. They do not have the complete flavor profile of ground spices, but they are used where a strong aromatic effect is desired. Essential oils are used at a very low level of

0.01% to 0.05% in the finished product. They can be irritating to the skin, toxic to the nervous system if taken internally (by themselves), and can cause allergic reactions and even miscarriages (Raghavan, 2007).

The essential oils in spices are generally composed of hydrocarbons (terpene derivatives) or terpenes (e.g., α-terpinene, α-pinene, camphene, limonene, phellandrene, myrcene, and sabinene), oxygenated derivatives of hydrocarbons (e.g., linalool, citronellol, geraniol, carveol, menthol, borneol, fenchone, tumerone, and nerol), benzene compounds (alcohols, acids, phenols, esters, and lactones) and nitrogen- or sulfur-containing compounds (indole, hydrogen sulfide, methyl propyl disulfide, and sinapine hydrogen sulfate). Terpene compounds are the major chemical components of most of the essential oils. Depending up on the molecular size, monoterpenes, diterpenes, triterpenes, and sesquiterpenes occur. Monoterpenes are the most volatile of these terpenes and constitute the majority of the terpenes in spices. Sesquiterpenes are most concentrated in ginger family (Raghavan, 2007).

The nonvolatile and volatile flavor components of spices, also referred to as oleoresins, are produced by grinding or crushing the spices, extracting with a solvent, and then removing the solvent. Oleoresins have the full flavor, aroma, and pungency of fresh or dried spices because they contain the high boiling volatiles and non-volatiles, including resins and gums that are native to spices. The nonvolatile components create the heat and or pungency of black pepper, mustard, ginger, and chile peppers. These components can be acid-amides, such as capsaicin in red pepper or piperine in black pepper, isothiocyanates in mustard, carbonyls such as gingerol in ginger, and thioethers such as the diallyl sulfides in garlic or onion (Raghavan, 2007).

The different pungent and or heat principles give different sensations e.g. spicy, hot, sharp, biting, or sulfury. The pungent sensation of onion or garlic is sulfury, while that of Jamaican ginger is spicy. Red pepper and white pepper do not contain much aroma because they have very little essential oils, whereas ginger, black pepper, and mustard contribute aromatic sensations with their bites because of a higher content of volatile oils. White pepper has a different bite sensation than black pepper because of their differing proportions of non-volatiles, piperine, and chavicine (Raghavan, 2007).

The taste of a spice such as sweet, spicy, sour, or salty, is due to many different chemical components such as esters, phenols, acids, alcohols, chlorides, alkaloids, or sugars. Sweetness is due to esters and sugars; sourness to organic acids (citric, malic, acetic, or lactic); saltiness to cations, chlorides, and citrates; astringency to phenols and tannins; bitterness to alkaloids (caffeine and glycosides); and pungency to the acid-amides, carbonyls, thio ethers, and isothiocyanates (Raghavan, 2007).

The ratio of volatiles to non-volatiles varies among spices causing flavor similarities and differences within a genus and even within a variety. Within the genus *Allium,* for example, there are differences in flavor among garlic, onions, chives, shallots, and leeks, which differ in this ratio. They vary depending upon the species of spice, its source, environmental growing and harvesting conditions, and storage and preparation methods. Even the distillation techniques can give rise to varying components—through loss of high boiling volatiles, with some components not being extracted or with some undergoing changes. Non-volatiles in a spice also vary with variety, origins, environmental growth conditions, stage of maturity, and postharvest conditions. For example, the different chile peppers belonging to the Capsicum group, such as habaneros, cayennes, jalapenos, or poblanos, all give distinct flavor perceptions, depending on the proportion of the different nonvolatiles, the capsaicinoids (Peter, 2001).

Spices can be used in foods as antioxidants. They help fight the toxins created by our modern world. Heat, radiation, UV light, tobacco smoke, and alcohol initiate the formation and growth of the free radicals in the human body. Free radicals damage the human cells and limit their ability to fight off cancer, aging, and memory loss. Many spices have components that act as antioxidants and that protect cells from free radicals. The chemical components responsible for antioxidant activity in ginger are gingerol and shogoal (Raghavan, 2007).

Cumin (*Cuminum cyminum*)

Cumin (*Cuminum cyminum*) is a flowering plant in the family Apiaceae, native from the east Mediterranean to East India. In India cumin is known in as ‘jeera’ or ‘jira’ and in Iran it is called ‘zira’. Indonesians call it ‘jintan’ (or jinten) and in China it is called ‘ziran’ but in Pakistan it is known as ‘zeera’. Cumin is a herbaceous annual plant, with a slender branched stem 20-30 cm tall. The leaves are 5-10 cm long, pinnate or bipinnate, thread-like leaflets. The flowers are small, white or pink, and borne in umbels. The fruit is a lateral fusiform or ovoid achene 4-5 mm long, containing a single seed. Cumin seeds are similar to fennel and anise seeds in appearance, but are smaller and darker in color. The English cumin was derived from the French cumin, which was borrowed indirectly from Arabic ‘Kammon’ via Spanish ‘comino’ during the Arab rule in Spain in the 15th century. The spice is native to Arabic-speaking Syria where cumin thrives in its hot and arid lands. Cumin seeds have been found in some ancient Syrian archeological sites. The word found its way from Syria to neighboring Turkey and nearby Greece most likely before it found its way to Spain. Like many other Arabic words in the English language, cumin was acquired by Western Europe via Spain rather than the Grecian route. Some suggest that the word is derived from the Latin ‘cuminum’ and Greek ‘kuivov’. The

Greek term itself has been borrowed from Arabic. Forms of this word are attested in several ancient Semitic languages, including 'kamunu' in Akkadian. The ultimate source is believed to be the Sumerian word 'gamun' (Zohary and Hopf, 2000). The use of cumin is very common in Indian and Pakistani foods. It is used to season many dishes, as it draws out their natural sweetness. It is traditionally added to curries, enchiladas, tacos, and other Middle-Eastern, Indian, Cuban and Mexican-style foods. It can also be added to salsa to give it extra flavor. Cumin has also been used on meat in addition to other common seasonings. The spice is extensively used in the cuisines of the Indian subcontinent. Cumin was also used heavily in ancient Roman cuisine (Peter, 2001; Raghavan, 2007).

The nutritional value of cumin seeds per 100 g includes Energy 370 kcal (1570 kJ), Carbohydrates 44.24 g, Dietary Fiber 10.5 g, Fat 22.27 g, Protein 17.81 g, Water 8.06 g, Thiamin (Vit. B1) 0.628 mg, Riboflavin (Vit. B2) 0.327 mg, Niacin (Vit. B3) 4.579 mg, Vitamin B6 0.435 mg, Vitamin C 7.7 mg, Vitamin E 3.33 mg, Calcium 931 mg, Iron 66.36 mg, Magnesium 366 mg, Phosphorus 499 mg, Potassium 1788 mg, Sodium 168 mg, Zinc 4.8 mg and other trace elements (U.S.D.A., 2008).

Cumin has high total dietary fiber content and the spent residue (after oils and nonvolatiles extraction) has been also found to contain high dietary fiber. Results show that the total dietary fiber content (TDF) of cumin is 59.0%, insoluble dietary fiber (IDF) 48.5%, and soluble dietary fiber (SDF) 10.5%, while the spent residue from cumin has been found to contain 62.1% TDF, 51.7% IDF and 10.4% SDF. The spent residue also contains 7.7% starch and 5% bound fat (Sowbhagya *et al.,* 2007).

Cumin essential oil contents

The most important chemical component of cumin fruits is essential oil content, ranging from 2.5% to 4.5% which is pale to colorless depending on age and regional variations. The ripe seeds of cumin are used for essential oil production, both as whole seeds or coarsely ground seeds. If freely alcohol-soluble oil is required, the whole seed must be used. Hydro distillation is used for essential oil extraction, producing a colorless or pale-yellow oily liquid with a strong dour. The yield for oil production varies from 2.5 to 4.5%, depending on whether the entire seed or the coarsely ground seed is distilled. The volatile oil should be kept in well-sealed bottles or aluminium containers (Peter, 2001).

Different studies have been conducted on the yield of cumin essential oil. Sowbhagya *et al.* (2008) evaluated the effect of size reduction and expansion on yield and quality of cumin (*Cuminum cyminum*) seed oil. For small batch size operations (200g), oil yield was found to be the same (3.4%) for both ground and flaked samples. However, in the operations of larger batch, flakes resulted in significantly higher (3.3%) oil yield as compared to ground samples (2.8%) indicating the advantage of flaking over grinding. Aqueous portion of the distillate in both cases had equal proportion of volatile oil (0.2%). Flavor profiles of the volatile oils revealed that retention of lower boiling terpene compounds and character impact compound, cuminaldehyde were higher in oil obtained from flakes as compared to powder.

Li *et al.* (2009) explored the extraction of essential oil from *Cuminum cyminum* seeds using a combination of organic solvent with low boiling point and steam distillation. The effect of different parameters, such as particle size, temperature and extraction time, on the extraction yield was investigated. The temperature had the largest effect on the yield of the extract (oleoresin), followed by extraction time and particle size. Essential oil of *C. cyminum* seeds obtained by supercritical fluid extraction (SFE), hydrodistillation (HD), combination technology of organic solvent with low boiling point and steam distillation (OS-SD) were further analysed by GC-MS detection to compare the extraction methods. Forty-five compounds in the *C. cyminum* essential oil were identified, showing that the composition of the extraction by different methods was mostly similar.

The essential oil is responsible for the characteristic cumin odor. This odor and flavor is due principally to the aldehydes present. Studies of the chemical composition of cumin oil from different countries showed the presence of the following components: α-pinene (0.5%), Myrcene (0.3%), limonene (0.5%), 1-8-cineole (0.2%), p-menth-3-en-7-ol (0.7%), p-mentha-1, 3-dien-7-ol (5.6%), caryophyllene (0.8%), β-bisabolene (0.9%), β-pinene (13.0%), P-cymene (8.5%), β-phellandrene (0.3%), D-terpinene (29.5%), cuminic aldehyde (32.4%), cuminyl alcohol (2.8%), β-farnesene (1.1%) together with much smaller quantities of α-phellandrene, α-terpinene, cis and trans sabinene, Myrtenol, α-terpineol and phellandral (Peter, 2001).

Other studies show that cumin essential oil mainly contains monoterpene aldehydes. The major compounds include cumin aldehyde (p-isopropyl-benzaldehyde, 25 to 35%), terpinene (29.5%), α- and β-pinene (21%), ρ-cymene (8.5%), ρ-mentha-1,3-dien-7-al (5.6%), cuminyl alcohol (2.8%) and β-farnesene (1.1%). Furthermore perilla aldehyde, cumin alcohol, dipentene, and β-phellandrene are also present in cumin. In toasted cumin fruits, a large number of pyrazines have been identified as flavour compounds. Besides pyrazines and various alkyl derivatives (particularly, 2,5- and 2,6-dimethyl pyrazine), 2-alkoxy-3-alkylpyrazines seem to be the key compounds e.g. 2-ethoxy-3-isopropyl pyrazine, 2-methoxy-3-sec-butyl pyrazine, 2-methoxy-3-methyl pyrazine. A sulfur compound, 2-methylthio-3-isopropyl

pyrazine is also found. Cumin also contains 10% fixed oil (El-Hamidi and Ahmed, 1966; Raghavan, 2007).

In a study, the essential oil composition of cumin seeds after subjecting them to heating by microwaves and conventional roasting at different temperatures was studied. The conditions were standardized in both methods. The volatile oils distilled from these samples were analysed by GC and GC–MS. The results indicated that the microwave-heated samples showed better retention of characteristic flavor compounds, such as aldehydes, than did the conventionally roasted samples (Behera *et al.,* 2004).

Jalali-Heravi *et al.* (2007) used Gas chromatography–mass spectrometry to characterize the essential oil components of Iranian cumin. A total of 19 components were identified by direct similarity searches for cumin oil. This number was extended to 49 components, with the help of chemometric techniques. Major constituents in cumin are gamma-terpinene (15.82%), 2-methyl-3-phenyl-propanal (32.27%) and myrtenal (11.64%).

In addition to volatile oil cumin also contains nonvolatile chemical components including tannins, oleoresin, mucilage, gum, protein compounds and malates. The oleoresins are obtained by subjecting the ground cumin to different organic solvents such as n-hexane, ethanol, methanol etc. The extract obtained is then subjected to rotary evaporation to remove the solvent (Peter, 2001).

Kanakdande *et al.* (2007) studied the microencapsulations of cumin oleoresin by spray drying using gum arabic, maltodextrin, and modified starch and their ternary blends as wall materials for its encapsulation efficiency and stability under storage. The microcapsules were evaluated for the content and stability of volatiles, and total cuminaldehyde, γ-terpinene and p-cymene content for six weeks. Gum Arabic offered greater protection than maltodextrin and modified starch, in general, although the order of protection offered was volatiles>cuminaldehyde> p-cymene> γ-terpinene. A 4/6:1/6:1/6 blend of gum arabic/ maltodextrin/ modified starch offered a protection, better than gum arabic as seen from the $t_{1/2}$, i.e. time required for a constituent to reduce to 50% of its initial value. However protective effect of ternary blend was not similar for the all the constituents, and followed an order of volatiles>p-cymene> cuminaldehyde >γ-terpinene.

Antioxidative properties of cumin

Cumin has also been tested for its antioxidative properties. The total phenolic content of methanolic extracts of different cumin varieties (cumin, black cumin and bitter cumin) ranged from 4.1 to 53.6 mg/g dry weight. Cumin (*Cuminum cyminum*) methanol extract was found to contain a total phenolic content of 9 mg/g dry weight. It has been also shown that the methanolic extracts of cumin show higher antioxidant activity compared with that of the aqueous extract (Thippeswamy and Naidu, 2005).

In another study the antioxidant activity and the phenolic compounds of 26 spice extracts including cumin was assessed. Antioxidant activity was expressed as TEAC (mmol of trolox/ 100 g of dry weight). Cumin showed a value of 6.61 mmol of trolox/ 100 g of dry weight while the total phenolic content of cumin was 0.23g of gallic acid equivalent/ 100 g of dry weight (Shan *et al.* 2005).

The antioxidant capacity of cumin (*Cuminum cyminum*) has been tested on Fe^{2+} ascorbate induced rat liver microsomal lipid peroxidation, soybean lipoxygenase dependent lipid peroxidation and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging methods. The total phenolic content of methanolic extract of cumin was 9 mg/ g dry weight. IC_{50} values of the methanolic extract of cumin seeds were 1.72 ± 0.02, 0.52 ± 0.01 and 0.16 ± 0.30 on the lipoxygenase dependent lipid peroxidation system, the DPPH radical scavenging system and the rat liver microsomal lipid peroxidation system, respectively. The data also showed that cumin is a potent antioxidant capable of scavenging hydroxy, peroxy and DPPH free radicals and thus inhibits radical-mediated lipid peroxidation (Thippeswamy and Naidu, 2005).

Damasius *et al.* (2007) assessed the antioxidant properties of aqueous and ethanol extracts of cumin (*Cuminum cyminum* L.). Antioxidant activity of cumin ethanol and aqueous extracts was measured in DPPH and ABTS radical scavenging reaction systems and depended on extract concentration. The aqueous extract of cumin showed higher DPPH radical scavenging activity while in ABTS reaction system the ethanol extract exhibited higher activity than the aqueous extract.

Lee (2005) studied the therapeutic properties of cumin. He evaluated the inhibitory activity of *Cuminum cyminum* seed-isolated component against lens aldose reductase and R-glucosidase isolated from Sprague-Dawley male rats and compared to that of 11 commercially available components derived from *C. cyminum* seed oil, as well as quercitrin as an aldose reductase inhibitor and acarbose as an R-glucosidase inhibitor. The biologically active constituent of *C. cyminum* seed oil was characterized as cuminaldehyde by various spectral analyses. The IC_{50} value of cuminaldehyde is 0.00085 mg/mL against aldose reductase and 0.5 mg/mL against R-glucosidase, respectively. Cuminaldehyde was about 1.8 and 1.6 times less in inhibitory activity than acarbose and quercitin, respectively. The author concluded that cuminaldehyde may be useful as a lead compound and a new agent for antidiabetic therapeutics.

Conclusion

The overall evaluation of this study concludes that the cumin have a good antioxidant potential. The essential oil of spices showed appreciable amounts of antioxidant compounds having high antioxidant activity and its nonvolatile extracts also have good inhibition properties against the free radicals. Methanol extracts were found to have better antioxidant action than the n-hexane extracts. There is also a good correlation between the total phenolic content and antioxidant activities of the nonvolatile extracts. So this study concludes that cumin have good antioxidant potential and this spices can be used to produce novel natural antioxidants as well as flavoring agents that can be used in various food products.

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Effect of extruder variables on chemical characteristics of Maize (*Zea mays.* L) extrudates

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Abstract

Extrusion cooking is progressively more being used for production of a wide range of snack foods and breakfast cereals. In this study two maize grits samples were obtain from dent maize (R-2303) and a flint type of maize (R-2207). Different extruder variables applied were temperature, screw speed and moisture content. T_6 of (R-2207) was found most suitable among all treatments (T_6 150 ºC, 170 r.p.m, 18% m.c.). Temperature, screw speed and moisture content, these extruder variables considerably affected the product quality. For R-2303 and R-2207 corn extrudates, T_6 and T_5 obtain the least moisture content of 3.65 % and 3.90 % correspondingly, while T_0 was at bottom obtaining 5.23 % moisture content. While, T_6 obtained the least amount of ash content of 0.40 % and 0.33 % respectively, while T_0 and T_4 was at bottom obtaining 0.45 and 0.43 % ash content. It is obvious from the results that T_6 of R-2207 positioned at the first for its lower fat level that possibly will be due to high temperature. R-2303 and R-2207 maize extrudates, T_6 got the least protein content of 8.96 %, while T_0 was at underside obtaining 8.36 % protein content .The protein content for uncooked corn grits of R-2303 and R-2207 was 9.22, 8.70 % correspondingly. Extrusion does not extensively have an effect on mineral composition of maize extrudates, except for iron. Iron level of the grits increased following processing into extrudates that may be the result of metallic pieces, mainly screws, of the extruder. In ending, extrusion cooking enhances apparent absorption of most minerals.

Keywords: Maize, extrusion, protein, fat, minerals, moisture, Temperature, screw speed

Introduction

Millions of people, mostly in the developing countries, meet up their protein and calorie necessities from maize. The maize grain accounts for about 15 to 56% of the total daily calories in diets of people in about 25 developing countries where animal protein is scarce and expensive and consequently, unavailable to a vast sector of population (Prasanna *et al.*, 2001).

Maize is also of significant importance in the countries like Pakistan, where rapidly increasing population has already outstripped the available food supplies (Shah *et al.*, 2003). It will be cultivated on an area of 10, 15,000 hectares with production 33, 18,000 tons during the 2008-2009. The production will be increased by 7.3% than that of previous year (GOP, 2009). Extrusion technology has been around for a long period of time in one form or another in many food industries Extrusion cooking is a quite latest form of food processing. Forcing material in the course of a hole is the process of extrusion. During the 1930s heat was provided to the barrel include the screw; puffed corn curl snacks resulted product. The pressure produced as the ingredient moved beside the screw; this, collectively with the heating under pressure caused the corn to expand on exiting the dies. Since extrusion cooking processed more types of food, extruders became more particular for food use.

Most extruders work as heat exchangers and they also shape food products. Mixing, dehydration and pasteurization and sterilization are extra unit operations that usually take place during extrusion. Aside from thermal decomposition of nutrients, the shear force that produced within the extruder barrel can spoil food chemical composition.

Food Product quality characteristics can differ significantly depending on the extruder type of the extruder, configuration of screw, feed moisture contents and temperature of the barrel session, speed of screw and feed rate Extrusion cooking produced safe and sound, frivolous, shelf-stable food that can be pile up for use throughout food crisis and natural disasters. Simple single screw extruders are quite economical and easy to maintain so these machines used in under developed countries for the manufacturing of weaning and new foods (Qingbo *et al.*, 2005). Extrusion cooking can formulate customary product to be more suitable in the swift changing society.

Extrusion cooking process, even though flexible, is multifaceted since it necessitates close control of many variables. Any variation in composition of feed, affect by the nature of constituent as well as the quantity of every constituent, and any extrusion process variable that affects physical or chemical conversion of macromolecules during extrusion can affect extrusion functionality and quality characteristics of the extrudates. consequently, new sets of optimal or advantageous processing conditions (temperature, screw speed, moisture content) are mandatory for each set of new conditions, and these are be obliged to be determined empirically as there is no completely developed process to tell the consequence of existing process variables on different complex raw materials and their combination (Smith, 1976). Starch is generally the key food ingredient in extruded foods such as breakfast cereals, snacks and

weaning foods. Humans do not readily digest native starch. Distinct several thermal processes, extrusion cooking gelatinizes starch fairly low (12-22%) moisture level. Increased temperature of barrel, shear force and pressure in extrusion raise the level of gelatinization of starch, but lipids, sucrose, dietry fiber and salts can impede gelatinization (Jin t al, 1994). While full gelatinization may not occur through extrusion, digestibility is frequently enhanced (Wang, S et al, 1993). Extrusion may progress protein digestibility by denaturing proteins, revealing enzyme-accessible sites.

Globally the snack food industry is fetching up bigger and more imperative every day. In the last ten years, revolution in way of living and eating habits have escort to an ongoing enhance in demand for snack foods. The way of snacking in various countries can be influenced by numerous aspects like as the diversity in lifestyle in every area, the economic conditions, and public accessibility of existing observation on nutritional subject. Snacks can supply a better dietary intake of essential amino acids and other food nutrients for developing countries. Extrusion cooking is preferable over other food-processing methods due to of constant process with high efficiency and important nutrient preservation, due to the high temperature and short cooking time required (Guy, 2001).

There is a need to make use of locally-grown crops to produce of suitable local recipes in the under developed countries has been tense by international agencies as the most efficient channel for addressing depending world food harms (Iwe *et a!.* 2001). Maize is the cereal of major importance in the developing world and has the maximum genetic production potential of all the cereal crops. Designing extruded snack foods today can be a difficult process to meet up varying consumer's tastes and demands. The consumer's requirement for "good for your health" and "distinctive flavor" extruded snacks leads to the indefinable exploration for something exceptional that also demand to extensive range of peoples. Consequently the current study has been designed to investigate the effect of different extruder variables temperature, screw speed and moisture content on the chemical and mineral profile of two different maize cultivars.

Materials and methods

The current research was performed to determine the suitability of maize cultivars for extrusion cooking and effect of different extrusion parameters on quality of product. The study proposed in this manuscript was conducted at the "Extrusion Center", National Institute of Food Science and Technology, University of Agriculture, Faisalabad.

Procurement of raw material

Two commercial maize cultivars namely hybrid R-2303 yellow dent corn and R-2207 White flint corn were procured from Rafhan Maize Faisalabad.

Cleaning

Maize grains were cleaned physically to eliminate dust particles, broken seeds, seeds of other crops and other contaminants like as weeds and metals.

Preparation of raw material

Two commercial maize cultivars namely hybrid R-2303 yellow dent corn and R-2207 White flint corn were milled to obtain maize grits according to a milling diagram proposed by Robutti *et al.,* (2002).

Extrusion process

The maize cultivars were extruded through a single-screw, short barrel (90 mm) snack food extruder (Extru-Tech, Inc.Model # KN, Sabetha, Ks 66534). The extruder was fixed with a 2 start screw and a 2-hole die with 4mm orifice. The prepared raw material, maize grits were fed into the extruder at the rate of 20 Kg /h using a mass flow feeding device. The extruder was run at different barrel temperatures ranges between 110 and 150 °C. Energy consumption during extrusion differs among the grits extruded in the research. Extrusion cooking was initiate to be a high-temperature, short-time process in which moistened, extensive, starchy and protinacious food materials are plasticized and cooked in a tube by a permutation of moisture, pressure, temperature and mechanical shear .The plastic foam set quickly as it left the die, forming a light, crispy, firm material alike to various well recognized snack foods. As the extrudates come out from the die, it was cut into 10–12 mm long snack-like extrudates with a revolving knife. Promising extrudates were instantly spread on the laboratory table at room temperature for 4 h before drying in the oven at 60 °C for 10 h to attain a final moisture content of 4-5% and stored in plastic bags at room temperature for more analysis.

Chemical analysis of maize grits and extrudates

Chemical analysis of maize grits and extrudates were carried out by following the methods illustrated in (AACC, 2000).

Moisture Content

The moisture content in maize grits and grounded extrudates was estimated by drying samples in a hot air oven at a temperature of 100± 5 °C until to a constant weight. Moisture contents was determined by following the method no 44-15A of AACC (2000).

The extruder variables applied in this study are given in Table1

Table 1: Detail of different treatments (temperature, screw speed and moisture content) during extrusion for two maize cultivars (R-2303 and R-2207)

Different treatments	Temperature °C	Screw speed (R.p.m)	Moisture content %
T0	105	110	18
T1	130	120	20
T2	135	120	17
T3	135	140	20
T4	150	170	19
T5	150	150	20
T6	150	170	18

Ash Content

The amount of ash present in maize grits and extrudates was determined as a total inorganic material by following the method no. 08-01 of AACC (2000).

Crude Protein

The amount of nitrogen in the samples was determined by using Kjeldahl method as described in AACC (2000). The crude protein percentage was estimated by multiplying nitrogen (%) with a factor 6.25 as given below:

Crude Protein = Nitrogen (%) X 6.25

Crude Fat

The crude fat content present in maize grits and extrudates of each maize cultivar was calculated by running oven dried samples through Soxhlet apparatus for 2-3 hours using petroleum ether as a solvent according to the procedure described in AACC (2000) method no. 30-10.

Mineral contents

Minerals (Fe, Zn, Mn and Cu) present in the raw maize grits and extrudates was determined by digesting 0.5 g sample in concentrated HNO_3 at a temperature of 85 °C and than $HClO_4$ at a temperature of 180 °C until 1-2 ml of digested sample remained. The digested sample was then filtered and volume was made to 25 ml. The digested samples were tested for minerals contents by using Atomic Absorption Spectrophotometer. Na, K and Ca were estimated by using the flame photometer according to the procedure described in AACC (2000).

Statistical Analysis

Analysis of variance (ANOVA) was used to establish significant differences among the eight treatments for two corn varieties. Statistics were reported at a significance level of 0.05. Duncan's Multiple Range Test (DMR) was applied for pair wise comparison of treatment means (Steel *et al.,* 1997). Each treatment was replicated three times for all the analysis.

Results and discussion

Chemical analysis of maize grits and extrudates

Maize grits and extrudates from two maize cultivars were evaluated for their chemical properties.

Moisture content of maize grits and extrudates

Nonaka (1997) reported that the moisture content of a food is one warning of a food's stability and value. Wetness is a positive sensory characteristic in baked products for the reason that it is identical with a soft, affectionate product. On the other hand, too much moisture endorses microbial development. The moisture content for raw corn grits of R-2303 was 10.60 % and for R-2207 was 9.72 % presented in table No. 2 was considerably superior than the value examine for maize extrudates. The effect of this study are in secure conformity with the conclusion of Badrie and Mellowes (1991) who discover that increasing extrusion temperature from 105 to 150° C reduced moisture level in extrudates, this was perhaps due to an raise in starch degradation. In the same way, moisture content first increased at low screw speed and then declined with elevated screw speed 120 to 170 r. p. m. It is evident from results that maize variety significantly affects the moisture of the maize extrudates. The treatments also significantly affect the moisture of maize extrudates. The interactive affect of maize variety and extrusion temperature also found significant with respect to moisture. The mean values of moisture contents of maize extrudates are given in table no.3. It is evident from results that moisture significantly with treatments as a function extruder temperature, screw speed, and moisture content. The moisture observed in maize extrudates is

It is obvious from the results that judges ranked T_6 at the first and T_0 at the last position. For R-2303 and R-2207 corn extrudates, T_6 and T_5 obtain the least moisture content of 3.65 % and 3.90 % correspondingly, while T_0 was at bottom obtaining 5.23 % moisture content.

Table 2: Moisture, ash, fat and protein contents of maize grits.

Maize Cultivar	Moisture %	Ash%	Fat%	Protein%
R-2303	10.60 ab	0.46 a	1.01 a	9.83 a
R-2207	9.72 b	0.42 ab	0.78 b	8.96 b

Table 3: Effect of extrusion variables on the moisture contents of extrudates of two maize cultivars.

Treatments	R-2303	R-2207	Mean
T_0	6.20a	5.23b	5.72
T_1	5.90a	4.74c	5.32
T_2	5.65b	4.76c	5.20
T_3	5.40bc	4.54cd	4.97
T_4	5.26bc	4.07d	4.66
T_5	4.55d	3.90de	4.22
T_6	4.15e	3.65e	3.90
Mean	5.30a	4.41b	

(T_0 105ºC, 110 r.p.m, 18% m.c), (T_1 130 ºC,120 r.p.m, 20% m.c.) (T_2 135 ºC,120 r.p.m, 17% m.c.) (T_3 135 ºC, 140r.p.m, 20% m.c.) (T_4 150 ºC, 170 r.p.m, 19% m.c.)
(T_5 150 ºC, 150 r.p.m 20% m.c.) ($T_6$150 ºC, 170 r.p.m, 18% m.c.)

Table 4: Effect of extrusion variables on the ash contents of extrudates of two maize cultivars.

Treatments	R-2303	R-2207	Mean
T_0	0.45a	0.48bc	0.465
T_1	0.43ab	0.54a	0.485
T_2	0.40ab	0.52ab	0.42
T_3	0.40ab	0.50ab	0.45
T_4	0.43ab	0.46ab	0.445
T_5	0.37ab	0.43ab	0.40
T_6	0.33b	0.40ab	0.365
Mean	0.401b	0.475a	

(T_0 105ºC, 110 r.p.m, 18% m.c), (T_1 130 ºC,120 r.p.m, 20% m.c.) (T_2 135 ºC,120 r.p.m, 17% m.c.) (T_3 135 ºC, 140r.p.m, 20% m.c.) (T_4 150 ºC, 170 r.p.m, 19% m.c.)
(T_5 150 ºC, 150 r.p.m 20% m.c.) ($T_6$150 ºC, 170 r.p.m, 18% m.c.)

Ash content of corn grits and extrudates

It is evident from results that maize variety had significantly affected the ash of the maize extrudates. The treatments also significantly affect the ash of maize extrudates. The interactive affect of maize variety and extrusion temperature also found non significant with respect to ash. The mean values of ash contents of maize extrudates are given in table no.4. It is evident from results that ash significantly differs with treatments as a function extruder temperature, screw speed, and ash content. The ash content for raw maize grits of R-2303 was 0.46 % and for R-2207 was 0.42 % 2was considerably (P <0.5) superior than the value examine for maize extrudates It is obvious from the results that judges ranked T_6 at the first and T_0 at the last position, when averaged general means were study. T_4 and T_5 were also privileged by the judges.

All the treatments effects were significant among each other DMR test was used to study the variations in treatments and cultivars. For R-2303 and R-2207 maize extrudates, T_6 obtained the least amount ash content of 0.40 % and 0.33 % respectively, while T_0 and T_4 was at bottom obtaining 0.45 and 0.43 % ash content .

This indicates that extrusion cooking outcome in significant preservation of nutrients like definite minerals like as calcium, iron and zinc. This is accredited to a high-temperature, short-time process of extrusion cooking, in that way yielding an improved product. The results are in harmony with the research of Alonso *et al.* (2001).

Fat content of maize grits and extrudates

It is evident from results that maize variety had significantly affected the fat of the maize extrudates. The treatments affect is non significant of maize extrudates. The interactive affect of maize variety and extrusion temperature was also found non significant with respect to fat. The mean values of fat contents of maize extrudates are given in table no. 5. The fat content for raw maize grits of R-2303 was 1.01 % and for R-2207 was 0.78 % presented in table no. 2 was considerably superior than the value examine for maize extrudates. Study of variance reveal an extremely large variation among the effects of treatments and cultivars on fat content of extrudates. It is obvious from the results that T_6 of R-2207 positioned at the first for its lower fat level that possibly will be due to high temperature. R-2303 and R-2207 maize extrudates, T_6 obtain the lowest amount of fat content of 0.74 %, while T_0 and T_2 was at bottom obtaining 0.963 and 0.960 % fat content. T_6 at 18 % moisture content, 150 °C temperature and 170 r. p. m. screw speed of R-2207 present highest expansion and least bulk density value due to lower fat content in grits. The results of this study are in secure conformity with the result of Thewessen *et al.,* (2002) who initiate that growing the fat content during extrusion have a unenthusiastic effect on expansion of different extruded systems.

Lin *et al.* (1997) study that the adding of fat during extrusion reduces considerably the level of starch gelatinization, due to drop off of the barrel temperature caused by the oily effect. Fat also diminish starch alteration during extrusion by avoiding harsh mechanical collapse of the starch granules by rotating screw and preventing water from being absorbed by starch. Decrease starch alteration/gelatinization eventually results in decreased expansion.

Protein content of maize grits and extrudates

It is evident from results that maize variety significantly affected the protein of the maize extrudates. The treatments affect is non significant of maize extrudates. The interactive affect of maize variety and extrusion temperature also found non significant with respect to protein. The mean values of protein contents of maize extrudates are given in table no. 6.

The protein content for raw maize grits of R-2303 was 9.83 % and for R-2207 was 8.96 % presented in table no. 2 was considerably superior than the value examine for maize extrudates. Study of variance explain extremely significant difference for protein content be present among the cultivars and non significant among the treatments and relations of cultivars and treatments. It is apparent from the results that T_6 positioned at the first and T_0 at the very last position

R-2303 and R-2207 maize extrudates, T_6 got the least protein content of 8.96 %, while T_0 was at underside obtaining 8.36 % protein content .The protein content for uncooked corn grits of R-2303 and R-2207 was 9.22, 8.70 % correspondingly. T_6 at 18 % moisture content, 150 °C temperature and 170 r. p. m. screw speed of R-2207 show highest expansion and least bulk density worth due to inferior protein content in grits. The consequences of this research are in lock conformity with the conclusion Mathew *et al.* (1999) who initiate that the reduced in protein content in corn meal assistance extrudate expansion, due to the raise in starch content.

According to Li (1991) proteins cover an effect on expansion through their aptitude to have an effect on water circulation in the medium and during their macromolecular structure and conformation, which influence the extensional properties of the extrudates. In the current study, the expansion ratio decline and bulk density increased like in T_0, T_1 and T_3 as a result of increasing protein content and small temperature and screw speed. Protein itself had a lower puffing ability compared with starch.

Table 5: Effect of extrusion variables on the fat contents of extrudates of two maize cultivars

Table of Mean

Treatments	R-2303	R-2207	Mean
T_0	0.963a	0.78a	0.871
T_1	0.937c	0.778a	0.854
T_2	0.960a	0.78a	0.870
T_3	0.940abc	0.77b	0.850
T_4	0.890d	0.76b	0.825
T_5	0.850e	0.78a	0.810
T_6	0.830f	0.74c	0.785
Mean	0.90d	0.75c	

(T_0 105ºC, 110 r.p.m, 18% m.c), (T_1 130 ºC,120 r.p.m, 20% m.c.) (T_2 135 ºC,120 r.p.m, 17% m.c.) (T_3 135 ºC, 140r.p.m, 20% m.c.) (T_4 150 ºC, 170 r.p.m, 19% m.c.)
(T_5 150 ºC, 150 r.p.m 20% m.c.) ($T_6$150 ºC, 170 r.p.m, 18% m.c.)

Table 6: Effect of extrusion variables on the protein contents of extrudates of two maize cultivars

Treatments	R-2303	R-2207	Mean
T_0	8.36d	8.90bc	8.63
T_1	9.38a	8.89bc	9.13
T_2	9.37a	8.91b	9.15
T_3	9.36a	8.93ab	9.14
T_4	9.15b	8.91b	9.03
T_5	9.12bc	8.91b	9.000
T_6	9.10bc	8.95a	9.020
Mean	9.33ab	8.91b	

(T_0 105ºC, 110 r..p.m, 18% m.c), (T_1 130 ºC,120 r.p.m, 20% m.c.) (T_2 135 ºC,120 r.p.m, 17% m.c.) (T_3 135 ºC, 140r.p.m, 20% m.c.) (T_4 150 ºC, 170 r.p.m, 19% m.c.)
(T_5 150 ºC, 150 r.p.m 20% m.c.) ($T_6$150 ºC, 170 r.p.m, 18% m.c.)

Minerals in maize grits and extrudates

Even though mineral constituent correspond to a small fraction the composition of foods, they play key roles in food nutrition. In spite of the significance of minerals for health, comparatively little studies have observed mineral steadiness during extrusion because they are secure in other food processes. The results related to minerals (Na) present in maize grits has been shown in Table 7. It was clear from the data that Na content was not extensively influenced by extrusion cooking for diverse treatments. Na content of R-2303 grits was 1.44 mg/100g and R-2207 grits was 1.88 mg/100g. Results shown in Table 7 have compared the Na content of diverse treatments among which the highest level of Na was observed in T_0 (1.85 mg/100g) table no 9. Extrudates of treatment T_6 (1.30 mg/100g) table no 8 were lesser in Na content.

It was apparent from the data that K content was considerably influenced by extrusion variables The study of variance for K content of extrudates prepared by various treatments has been shown in Table 8 and 9. It was apparent from the data that K content was considerably influenced by extrusion variables for different treatments. K content of R-2303 grits was 85 mg/100g and R-2207 grits was 89 mg/100g. Results are presented in table 7 have compared the K content of various treatments among which the highest level of K was observed in T_6 (152.22 mg/100g). Extrudates of treatment T_4 (148.22 mg/100g) were inferior in K content.

The study of variance for Ca level of extrudates prepared by different treatments has been shown in Table 8 and 9. It was clear from the data that Ca content was extensively influenced by extrusion cooking for dissimilar treatments. Ca content of R-2303 grits was 2.95 mg/100g and R-2207 grits was 3.7 mg/100g. Results presented in Table 8 and 9 include the Ca content of various treatments among which the maximum level of Ca was seen in T_1 (3.85 mg/100g). Extrudates of treatment T_0 (2.95 mg/100g) were lower in Ca content.

Extrusion conditions of (120-170 r. p. m. screw speed, 105-150 °C exit temperature and 18-20% moisture raise the level of iron than the levels of iron in uncooked grits. It was obvious from the data that Fe content was drastically changed by extrusion cooking for different treatments. Fe content of R-2303 grits was 1.35 mg/100g and R-2207 grits was 1.4 mg/100g. The highest level of Fe was observed in T_6 (2.8 mg/100g). Extrudates of treatment T_2 (2.46 mg/100g) were inferior in Fe content.

The study of variance for Mn level of extrudates prepared by different treatments has been shown in Table no 8 and 9. It was apparent from the data that Mn levels were not much affected by extrusion cooking for different treatments. Mn content of R-2303 grits was 34 mg/100g and R-2207 grits was 32.11 mg/100g. Values given in Table 17 include the Mn content of various treatments among which the utmost level of Mn was observed in T_4 (36.00 mg/100g). Extrudates of T1 (36.83 mg/100g) were lesser in Mn content.

The consequences are in harmony with Camire (2000) who suggested that rise in intensity of minerals in extrudates that may be accredited to the accumulation of these minerals throughout water used during extrusion.

Extrusion can develop the absorption of minerals by dropping other aspect that reduces absorption negatively (Alonso *et al.*, 2001). Extrusion does not extensively have an effect on mineral composition of maize extrudates, except for iron. Iron level of the grits increased following processing into extrudates that may be the result of metallic pieces, mainly screws, of the extruder. In ending, extrusion cooking enhances apparent absorption of most minerals.

Table 7: Mineral contents of maize grits (R-2303 and R-2207)

Maize Cultivar	Na mg/100g	K mg/100g	Ca mg/100g	Fe mg/100g	Mn mg/100g
R-2303	1.44b	121.02b	2.93b	1.60b	33.09a
R-2207	1.88a	145.55a	3.05a	2.44a	31.13b

Table 8: Effect of extrusion variables on the mineral contents of the extrudates of the cultivar R-2303

Treatments	Na	K	Ca	Fe	Mn
T_0	1.43ab	115.66b	2.95e	1.75b	34.45b
T_1	1.44a	120.63b	3.15d	1.67bc	34.95b
T_2	1.44a	120.83b	3.15d	1.53c	35.45a
T_3	1.40b	124.75b	3.25b	160bc	32.96c
T_4	1.36c	125.05ab	3.45a	1.53c	36.00a
T_5	1.33cd	123.75b	3.10c	1.7b	36.00a
T_6	1.30de	126.11a	3.23b	1.8a	35.45a

(T_0 105ºC, 110 r.p.m, 18% m.c), (T_1 130 ºC,120 r.p.m, 20% m.c.) (T_2 135 ºC,120 r.p.m, 17% m.c.) (T_3 135 ºC, 140r.p.m, 20% m.c.) (T_4 150 ºC, 170 r.p.m, 19% m.c.)
(T_5 150 ºC, 150 r.p.m 20% m.c.) ($T_6$150 ºC, 170 r.p.m, 18% m.c.)

Table 9: Effect of extrusion variables on the mineral contents of the extrudates of the cultivar R-2207

Treatments	Na	K	Ca	Fe	Mn
T_0	1.85ab	150ab	3.70bc	2.50ab	32.11cd
T_1	1.80cd	150.7ab	3.85a	2.65ab	32.00cd
T_2	186a	151.5ab	3.64c	2.46b	32.53c
T_3	186a	151.25ab	3.76b	2.64ab	34.01a
T_4	1.79	149.31b	3.76b	2.7ab	33.25b
T_5	1.82c	148.22b	3.76b	2.75ab	33.00b
T_6	1.77e	152.22ab	3.31d	2.8a	33.95ab

(T_0 105ºC, 110 r.p.m, 18% m.c), (T_1 130 ºC,120 r.p.m, 20% m.c.) (T_2 135 ºC,120 r.p.m, 17% m.c.) (T_3 135 ºC, 140r.p.m, 20% m.c.) (T_4 150 ºC, 170 r.p.m, 19% m.c.)
(T_5 150 ºC, 150 r.p.m 20% m.c.) ($T_6$150 ºC, 170 r.p.m, 18% m.c.)

Conclusion

Extrusion is an ultimate technique for producing a variety of extruded products such as snacks and breakfast cereals. Extrusion processing conditions (moisture content, low residence time and low temperature) help advanced preservation of amino acids, high protein and starch digestibility, and reduce lipid oxidation, higher retention of vitamins and higher absorption of minerals. Extrusion processing parameter Screw speed, temperature and moisture content has considerable effect on the physical properties of extudates and sensory properties of maize extrudates. Extrusion cooking does not extensively influence mineral composition of maize extrudates, except for iron. Iron content of the grits increased after processing into extrudates. The method used in this study allowed us to end that the harder maize grits of R-2207 gives products with greater expansion values, elevated degree of cooking and lower mechanical resistance than the softer one of R-2303. Our findings give support to the fondness of the snack industry for harder-maize grits. There are many areas that require further research regarding extrusion and nutrition.

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