REG. No. 24918 / 64
ISSN: 0022 - 1155
CODEN: JFSTAB

JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS (INDIA)

Vol.34, No.3 May-June 1997

ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS (INDIA) MYSORE - 570 013

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JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Volume 34 Number 3 May/June 1997

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Journal of Food Science and Technology, devoted to original R & D contributions in all branches of Science, Technology and Engineering of Foods and Food Products, is a bimonthly publication of the Association of Food Scientists and Technologists (India), Mysore. Review papers are also published to a limited extent.

Manuscripts for publication, books for reviewing and matter concerned with advertisements in the Journal should be addressed to the Editor, Journal of Food Science and Technology, AFST (I), CFTRI Campus, Mysore-570 013, India.

The Instructions to Authors are published in the January-February issue of the journal. These are required to be followed *in toto*. No deviation is accepted.

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Methods for Assessing the Immunostimulating Properties of Dietary Lactobacilli - A Critical Appraisal

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Several species of lactic acid bacteria are known as therapeutic agents since long. Consumption of fermented milks, containing dietary lactobacilli, confer positive influences on the gastro-intestinal microecology and hence they have been used in the treatment of a variety of GI-tract disorders. Dietary lactobacilli have also been found to have hypocholesterolemic and antitumour properties. In the last couple of decades, interesting observations on their immunomodulatory influences in several types of hosts have been reported. Selected species of lactobacilli play positively with host's defence mechanisms, which may involve checking the translocation of invading bacteria and development of specific and non-specific immune responses. Several *in vitro* and *in vivo* studies are conducted on experimental animals, but very limited work involving *in vivo* human studies have been done on this very interesting aspect of lactobacilli. This review critically analyses the work done so far in the area and will help in planning the future strategies for commercially and technologically exploiting this very important beneficial activity of lactobacilli.

Keywords : Immune response, Dietary lactobacilli, *Lactobacillus acidophilus*, Antitumour activity, Fermented milk.

Milk, nature's most complete food, is the gift to us from God. But, the fermented milk is one step ahead, because of its additional nutritional, therapeutic and preservative potentials. Fermentation has been the first food preservation technique employed by man from time immemorial. It has played many important roles in human nutrition. Interest in the role of lactobacilli in human health goes back atleast as far as 1908, when Eli Metchnikoff suggested that man should consume milk fermented with lactobacilli to prolong life (Metchnikoff 1908). His theory was that the lactobacilli would displace/prevent the microorganisms occurring in the intestinal tract, which otherwise reduce the life span by producing toxins.

Lactobacilli are Gram-positive, rods, typically non-motile, non-sporulating, micro-aerophillic, catalase negative microorganisms, which are widely distributed in nature and are easily isolated from mucous surfaces of mammals, green plants, milk and fermented foods (Sharpe 1979; Kandler and Weiss 1986). In human, lactobacilli are found in the mouth, lower intestine and vagina (Sandine et al. 1972; Sharpe 1979; Khedekar et al. 1990). Lactobacilli are also exploited (Kim 1988) for the manufacture of fermented milks like *dahi*, yoghurt, *acidophilus* milk, *kumiss* and *bioghurt* for human consumption (Lang and Lang 1975; Sellars 1991; Prajapati 1995) (Table 1). These products supply sufficient numbers of live dietary lactobacilli, which should be consumed live to achieve maximum therapeutic benefits (Puhan and Zambrini 1992). Many other dried foods containing live lactobacilli like *Malyukta, Malysh, Acidophilus* banana powder and *Acidophilus* malt food are also reported (Korobkina et al. 1981; Shah et al. 1986; Prajapati et al. 1987).

During fermentation of milk, lactobacilli are known to produce several antimicrobial compounds like lactic acid, acetic acid, hydrogen peroxide and bacteriocins, which are effective against several intestinal pathogens (Dave and Prajapati 1994) and therefore, consumption of these bacteria has been found to be useful in controlling a variety of intestinal disorders (Alm 1991). They have also been found useful in recolonization of intestine after heavy antibiotic treatment (Sandine 1979) and as a source of beta-galactosidase for lactose intolerants (Gilliland 1985). Besides, they are effective in hypercholesterolemia (Gilliland et al. 1985), hepatic encephalopathy (Read et al. 1966) and tumorigenesis (Friend and Shahani 1984). All these therapeutic benefits have been reviewed extensively. (Friend and Shahani 1984; Gilliland 1989; Alm 1991; IDF 1991).

During recent years, *in vitro* as well as *in vivo* interactions between dietary lactobacilli and immuno-competence has been studied well. A number of these studies have shown that lactobacilli, not only constitute an integral part of the host's gastrointestinal microecology (Yuguchi et al. 1992), but also play an important role in the host's immunoprotective system by increasing specific and non-specific immune mechanisms (Perdigon et al. 1987; Paubert-Braquet 1992). Colonization of the gut by lactobacilli has consistently been shown

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TABLE 1. FERMENTED MILKS CONTAINING DIETARY LACTOBACILLI

Name	Physical state	Culture(s) used	Acidity, % L.A.	Country in which popularly used	References
Acidophilus milk	Liquid/ gel	*Lb. acidophilus*	0.9-1.5	Europe, North America	Lang and Lang (1975) Gandhi and Nambudripad (1979)
Acidophillin	Liquid	*Lb. acidophilus* *Lc. lactis*, Kefir grains	0.7-1.2	Russia	Land and Lang (1975) Sharma and Gandhi (1981)
Acidophilus paste	Paste	*Lb. acidophilus*	1.6-1.8	Russia, Japan	Koroleva (1982)
Acidophilus-yeast milk	Gel	*Lb. acidophilus*, Lactose/sugar fermenting yeast	1.0	Russia	Lang and Lang (1975)
ACO-yoghurt	Gel	*Lb. acidophilus*, *Str. thermophilus* and *Lb. bulgaricus*	0.9-1.4	Switzerland	Lang (1980)
Bioghurt	Gel	*Lb. acidophilus*, *Str. thermophilus*	0.9-1.3	Germany	Kulpsch (1983)
Bulgarian butter milk	Liquid	*Lb. bulgaricus*	1.0-2.3	Europe, Balkan	IDF (1988)
Dahi	Gel	*Lc. lactis*, and its subspp. *Str. thermophilus*, *Lb. bulgaricus*	0.8-1.2	India, Pakistan Bangladesh	Laxminarayana (1984) Garg (1988) Prajapati (1995)
Kefir	Liquid	*Lb. casei*, *Lc. lactis* *Lb. acidophilus*, *Lb. bulgaricus*, *K. fragills*, *Candida kefir* (Kefir grains)	0.6-1.0	Middle East North Africa Russia	Koroleva (1982) IDF (1988)
Kumiss	Liquid	*Lc. lactis*, *Lb. bulgaricus* *C. kefir* or *Kluy. lactis*	0.6-0.8	Russia, Europe, Middle East East Asia	Koroleva (1982), IDF (1988)
Yakult	Liquid	*Lb.casei* (Shirota) and *Lb. acidophilus*	0.9-1.2	Japan	Anon (1971)
Yoghurt	Gel/ stirred	*Str. thermophilus* and *Lb. bulgaricus*	0.9-1.3	Worldwide	Tamime and Robinson (1985)

to stimulate the immune system and increase the host's resistance to infections (De Simone 1986). The information on this highly potential beneficial role of lactobacilli is scattered. Critical appraisal is of socio-economic and industrial importance. This review is an effort in this direction.

The immune system

The mechanism by which the body recognizes accurately and specifically the foreign antigens and eliminates them is called immune system. It consists of a number of organs and different types of cells. The organs of immune system are bone marrow, thymus, spleen, Peyer's patches and lymph nodes (Perdigon et al. 1995a). The immune system is commonly likened to an army and its various cells to soldiers. Their primary duty is to seek out and destroy or eliminate the invaders to protect our body (Young and Cohn 1988).

All these cells develop from a kind of master cell, called hematopoietic (blood-forming) stem-cell and initially appear in the human embryo in the yolk sac (Golde 1991). They, then, migrate to liver, as the foetus develops. Blood cells are created in the liver of the foetus, but blood is normally produced solely in the bone marrow shortly after birth (Golde 1991). These stem cells differentiate to form several types of blood cells, participating in immunity (Golde 1991; Nossal 1993). These cells, in general, are called leucocytes or white blood cells, which include granulocytes, monocytes and lymphocytes (Golde and Gasson 1988). The granulocytes are sub-divided into 3 groups called neutrophils, eosinophils and basophils. The neutrophil is essential in the host's defence against bacteria and some fungi, the eosinophil has a role in defending against parasites, such as worms and protozoans, while the function of basophil is less well understood. Monocytes (and related cells called macrophages) are crucial in the defence against intracellular parasites, such as viruses and certain bacteria. Lymphocytes help in recognizing and

destroying many types of pathogens. The T-lymphocytes give cell mediated immunity, while ß- lymphocytes give humoral (antibody defences) immunity (Golde and Gasson 1988).

Several factors may decrease the immune function, including exposure to UV light (Donawho and Kripke 1991), cigarette smoking (Bendich 1993), infection with viruses such as HIV (Greene 1993) and aging (Visek 1990, Goodwin and Burns 1991). Poor living conditions and malnutrition cause diminished resistance (Bellanti and Kadlec 1985). Skin and mucous membranes in our body act in non-specific immunity by providing a physical barrier to invasion. Any damage to these barriers also decreases immunity (Bellanti and Kadlec 1985). Microbial factors like the type and number of microflora on internal and external surfaces of the body also affect the immune function significantly (Bellanti and Kadlec 1985; Yuguchi et al. 1992).

The mechanism of host's defence that keeps microbial parasites in check is called, immune response. The host's immune response can be divided into two basic categories (Compos et al. 1993); i.e., innate (non-specific) and adaptive (specific), which differ in the mode of induction, antigen specificity and effector components. In disease situations, innate responses can be considered to be the first line of defence and are mainly mediated by macrophages, neutrophils and natural killer cells or by soluble components, such as complement and acute phase proteins. Adaptive responses are important for recovery and prevention of re-infection and are mediated by T-cells (lymphokine producers) and ß-cells (antibody producers) (Campos et al. 1993). Certainly, coordination exists between these two arms of the host response (innate and adaptive). However, certain cytokines can be associated primarily with one or the other type of response (Campos et al. 1993).

The invading microorganisms in extracellular environment are destroyed by phagocytosis (Paul 1993), while capsulated microorganisms like *Pneumococci* are destroyed after attachment with specific antibodies, thereby facilitating phagocytosis, called opsonization (Paul 1993). Parasites and intracellular organisms like *Leishmania* and *Mycobacteria*, which are able to survive even within the phagosomes (vacuoles in macrophage) are destroyed with the help of Class-II-MHC proteins and CD4-T-cells (Golde and Gasson 1988; Paul 1993), while viruses are tackled by Class-I-MHC proteins and CD8-T-cells (Paul 1993).

Immunostimulatory effects of dietary lactobacilli

Dietary lactobacilli are consumed through oral route and hence the mechanisms operating in the host, when an antigen is entering through oral route, become operative. Initially, this involves release of cytokines by lymphoid cells associated with mucosa, which interacts with the antigen (Perdigon et al. 1995a). Then, systematic immune response is exhibited in several ways. The discussion below represents the work done so far in these areas.

Mucosal immunity: The skin and mucosa membranes constitute the surface of the host that has some means of getting rid of the microorganisms, with the result that only certain specially adopted microorganisms can survive. The metabolic activities of such adopted flora are important in preventing the establishment of pathogenic microorganisms (Berg 1992).

The bacteria constituting the microflora, may translocate through the gastroenteric barrier to reach to the lymphatic organs (Peyer's patches, mesentric lymph nodes) and other organs (liver, spleen, blood) (De Simone et al. 1989b). This phenomenon is observed with Gram-negative bacteria and facultative anaerobes (i.e., *Escherichia coli*) more frequently than it is with obligate anaerobes and Gram-positive organisms (Berg 1992; De Simone et al. 1989b). Translocation of pathogens through this barrier is considered as either a deficiency of the mucosal barrier or the immune system or both (Berg 1983). It has been reported that the presence of fermented milk containing live lactobacilli can check the translocation of undesirable bacterial forms (IDF 1991; Aranaud-Battandier 1982).

In germ-free mice, Camaschella et al (1988) reported that *E. coli* could translocate through the lamina propria into peripheral organs and *E. coli* were found in lymph nodes of all animals (100% translocation), which had been treated with this microorganism. However, when the animals were pre-treated with yoghurt lactic bacteria (*Lactobacillus delbrueckii* subsp. *bulgaricus* or *Streptococcus thermophilus*), *E. coli* were absent in the lymph nodes or present in a significantly lower quantity, as compared to the number found in the monoassociated animals. In experiments on axenic mice, feeding of *Lb. bulgaricus* and *S. thermophilus* gave a reduction in the translocation of *E. coli* into the mesenteric lymph nodes of 70% and 50%, respectively (Bianchi Salvadori et al. 1988). They

ascribed this effect to the immune system stimulation by *Lb. bulgaricus*. In an *in vitro* study, De Simone et al (1989b) have reported that cells of *Lb. bulgaricus* exhibit marked cytoadherence to human lymphocytes. The % binding ranged between 30 and 40 at 1:200 lymphocyte : bacteria ratio. It bound CD4+ or CD8+ cells, while ß-cell binding was negligible.

Both cellular and humoral antimicrobial mechanisms also operate at the mucosal surface (Berg 1992). When a pathogen overcomes the non-specific host defence mechanism, the host activates specific defence mechanisms for producing antibodies, mainly S-IgA (secretory immunoglobulin A) (Aranaud-Battandier 1982). These antibodies can inhibit bacterial colonization by agglutinating microorganisms in the gut or by interfering with motility and blocking the bacterial surface structures responsible for adherence to the epithelium (Cantey 1978). The intestine is the largest immunological organ, which contains a large number of lymphocytes including ß-cells (Aranaud-Battandier 1982).

Nader et al (1993) fed mice for 8 consecutive days with fermented milk, containing *Lb. casei* and *Lb. acidophilus* ($1.5x10^8$ cells) and then challenged with *Listeria monocytogenes* and enteroinvasive *E. coli*. They found that the survival rates in control mice were 62% for *L. monocytogenes* and 83% for *E. coli*, while 100% protection was observed in treated mice for a period of 20 days. The levels of antipathogenic sera and intestinal antibodies in the latter cases were 2 to 4 times higher in treated mice. Several other workers (Perdigon et al. 1989, 1990, 1991a; Nader et al. 1992) also reported that in the mice, when fed fermented milks containing lactobacilli, the circulating and intestinal antibodies were increased at sufficient level that could protect the mice against different pathogenic challenges (Table 2).

After oral infection of mice with *S. typhimurium*, 100% survival was obtained in the group pre-treated by feeding *Lb. casei* and *Lb. acidophilus*-fermented milks for 8 days (Perdigon et al. 1990). A mixture of *Lb. casei* and *Lb. acidophilus*-fermented milk could markedly inhibit the colonization of liver and spleen with the pathogen and also showed increase in anti-salmonellae antibodies in serum and intestinal fluids (Perdigon et al. 1990).

Non-specific immune responses

Phagocytic activity: Macrophages play a central role in cell-mediated immunity as antigen presenting (Nossal 1993), tumoricidal (Golde and Gasson 1988) and microbiocidal cells (Roitt 1988). Perdigon et al (1987) reported that *in vitro* phagocytic activity of peritoneal macrophages increased by 3-4 times, than those of control mice, when *Lb. acidophilus* was injected intraperitoneally at a dose of 50 µg/d (equivalent to $1.2x10^9$ cells/g). Japanese researchers (Sato 1984; Yokokura et al. 1988) have conducted intensive research to study the protective effect of *Lb. casei* against intestinal infection caused by *Pseudomonas aeruginosa* and *Listeria monocytogenes*. The results indicated that *Lb. casei*

TABLE 2. EFFECT OF FERMENTED MILK ON THE LEVEL OF ANTIBODY IN CIRCULAR AND INTESTINAL FLUIDS OF MICE

Culture(s) used	Infectious organisms	Antibody level		References
		Circulating	Intestinal	
Lb. casei	*Salmonella typhimurium*	—	(3-4 fold)↑	Perdigon et al (1989)
Lb. bulgaricus	*E. coli*	(2-3 fold)↑ IgG	(2-3 fold)↑ IgG	Link-Amster et al (1989)
Lb. acidophilus + *Lb. casei*	*Salmonella typhimurium*	(5-fold)	(1-2 fold)↑	Perdigon et al (1990)
Lb. casei	*Salmonella typhimurium*	—	(S-IgA)↑	Perdigon et al (1991a)
	E. coli 0111K58	—	(S-Iga)↑	
Lb. casei	*Salmonella typhimurium*	↑	↑	Perdigon et al (1991b)
Lb. casei + *Lb. acidophilus*	*Shigella sonnei*	↑	↑	Nader de Macias et al (1992)
Lb. casei + *Lb. acidophilus*	*Listeria monocytogenes*	(3-4 fold)↑	(4-5 fold)↑	Nader de Macias et al (1993)
	E. coli	(5-fold)↑	(5-fold)↑	

↑ - Increased

S-IgA - Secretory Immunoglobulin-A

offered protection through activated macrophages. Goulet et al (1989) reported that the phagocytic activity of alveolar macrophages was significantly higher for mice fed with *Bifidobaterium longum, Lb. acidophilus, Lb. casei* subsp. *rhamnosus* and *Lb. helveticus*-fermented milks, than for the control groups fed with non-fermented UHT milk. No significant stimulation of phagocytic activity could be observed with streptococci-fermented milks (Goulet et al. 1989).

Moineau et al (1989) observed significantly higher ($P<0.05$) phagocytic indices for mice fed with *Lb. acidophilus* and *Lb. casei*, as compared to control group fed with milk. However, they did not observe significant differences in the levels of IgG and IgA in blood serum between experimental and control groups of mice. Murine macrophage cell line J774, when cultured in the presence of cell-free extracts of *Lb. acidophilus* and *B. longum*, resulted in induced morphological changes and significantly enhanced phagocytosis of inert particles or viable *Salmonella* in both the strains (Hatcher and Lambrecht 1993).

Effect of oral or intraperitoneal injection of *Lb. casei* CRL 431 and *Lb. bulgaricus* CRL 423 on the paritoneal macrophage activity was studied in mice (Perdigon et al. 1986a). The lower dose of *Lb. bulgaricus* for 8 days significantly increased the hydrolytic enzymes (ß-glucuronidase and ß-galactosidase) in macrophages, than the higher, one time dose. Similar results were obtained with *Lb. casei* also. They also indicated that either oral or intraperitoneal way was equally effective (Perdigon et al. 1986a). Intramuscular injection of immunopotentiator *Lb. casei* 9018 increased phagocytic function and suppressed the incidence of spinning disease in mice infected with *Mycobacterium fortuitum* complex (Saito et al. 1987). Intrerleukin-1 producing ability of macrophages was also increased by *Lb. casei* injections, causing possible enhancement of T-cell mediated immune response to mycobacteria (Saito et al. 1987).

During oral feeding of a mixture of *Lb. casei* and *Lb. acidophilus* to Swiss albino mice, Perdigon et al (1986b) observed an increase in both the *in vitro* phagocytic activity of peritoneal macrophages and the carbon clearance activity. Higher lymphocytic activity was also observed in mixture-fed mice, as compared to the control. Seven groups of 24 CD-1 mice were fed for 8 days with UHT milk fermented by different lactic acid bacteria. The percentage phagocytosis of pulmonary alveolar macrophages was significantly higher at 5 or 8 days of feeding in groups fed with *Lb. casei, Lb. helveticus* or *B. longum* (Moineau and Goulet 1991a). However, milk cultured with *Lb. bulgaricus* and 3 streptococci had no stimulatory effect on phagocytic activity (Moineau and Goulet 1991a).

Perdigon et al (1991b) observed increased phagocytic activities in mice fed heated or unheated yoghurt from second day of feeding onwards. However, the lymphocytic activity was not enhanced with any yoghurt. Heated yoghurt was not effective in preventing gastrointestinal infection induced by *S. typhimurium* and *E. coli*, but natural yoghurt, could prevent the infection at low infective doses

TABLE 3. *IN VITRO* STUDIES INDICATING IMMUNOMODULATING EFFECT OF LACTIC ACID BACTERIA

Cell types	Inducer	Effect	References
Kupffer cells, spleen, lung, peritoneal macrophages	*Lb. casei*	Produce cytotoxic factor	Hashimoto et al (1985)
Human peritoneal blood lymphocyte	Live yoghurt cells	3-4 times increase in gamma-interferon production	De Simone et al (1986)
	Heated yoghurt	No effect	
Human lymphocytes	*Lb. bulgaricus* *S. thermophilus*	Binding of 30 to 40% cells to bacteria	De Simone et al (1989b)
Human blood monocytes	Lysate of *Lb. bulgaricus*	Induction of membrane bound and cytoplasmic IL-1 and TNF-α	Popova et al (1993)
Murine macrophage cell line J774	Cell-free extracts of *Lb. acidophilus* *B. longum*	Enhanced phagocytosis of *Salmonella* or inert particles	Hatcher and Lambrecht (1993)
Human blood mononuclear cells	*Lb. bulgaricus* *S. thermophilus* *Lb. casei* *Lb. acidophilus*	Induced production of IL-1B, TNF - α and IFN - γ, but not IL - 2 and IFN - α	Pereyra and Lemonnier (1993)
Peyer's patch cells from normal and tumour induced mice	Kefir grain bacteria, its polysaccharides or protein fraction	Increased mitogenic activity of Peyer's patch cells	Furukawa et al (1996)

TABLE 4. *IN VIVO* EXPERIMENTS INVOLVING HUMAN SUBJECTS INDICATING IMMUNOMODULATORY EFFECT OF LACTIC ACID BACTERIA

Age group, years	Immunomodulator	Effect	References
21–56	Lyophilized *Lb. bulgaricus* and *S. thermophilus* + 200 g yoghurt	Increase in β lymphocytes, NK-cells, serum IgG and serum gamma-interferon	De Simone et al (1989 a, b)
Above 70	Lyophilized *B. bifidum* and *Lb. acidophilus*	Reduced colonic inflammatory infiltration, without altering T, B and Leu – 7+ cells percent and increase in β- cell frequency in peripheral blood	De Simone et al (1992)
Adult	Yoghurt	Increase in interferon production from blood mononuclear cells	Pereyra and Lemonnier (1993)
Adult	Cultured milk containing *Lb. acidophilus, bifidobacteria*	4-fold increase in specific serum IgA against *S. typhi* Ty 21a	Link-Amster et al (1994)
23–62	Cultured milk containing *B. bifidum, Lb. acidophilus*	Increased phagocytic activity but no modification of lymphocyte sub-populations	Schiffrin (1995)

of the said pathogens. Deodan, a lysozyme lysate of *Lb. bulgaricus* LB-51 has shown antitumour activity in mice and humans (Popova et al. 1993). The oral administration of deodan at the dose of 150 mg/kg daily (the recommended dose in human), in mice caused an increase of the spreading ability and phagocytic activity of peritoneal macrophages, which also resulted in slight increase in secretion of interleukin-1. This gave increased resistance to infection by *Klebseilla pneumoniae* and *L. monocytogenes* in mice. *In vitro* incubation of human blood monocytes with deodan, resulted in induction of membrane bound and cytoplasmic interleukin-1 and tumour necrosis factor-α (Popova et al. 1993) (Table 3).

NK-cell activity: Natural killer cell is also a lymphocyte, closely related to the cytotoxic T-cell. Its main targets are thought to be tumour cells, and perhaps also the cells infected by agents other than viruses (Young and Cohn 1988). When 20 healthy human volunteers were fed lyophilized yoghurt cultures for 28 days, the frequency of Leu 2-7+ cells and β-cells in blood progressively increased during the feeding period and thereafter within 60 days, the values came down to the initial level (De Simone et al. 1989a). (Table 4) NK-cell activity was also found to be enhanced by *Lb. plantarum* (Bloksma et al. 1981). When heat-killed *Lb. casei* was administered in mice, a marked augmentation of NK-cell activity in host spleen cells has been demonstrated (Kato et al. 1984). However, such a phenomenon was not noticed in mice given *Lb. fermentum*. They further reported that the potencies of NK-cell activity induced by *Lb. casei* in various strains of mice, did not coincide with the order of natural resistance to listerial infection, which indicated merely partial participation of NK-cells in protection of mice from listeriosis.

Specific immune responses

Cell-mediated immunity: The cell-mediated immunity plays an important role in protection against intracellular organisms and killing of viral infected cells (Roitt 1988). It does not produce humoral antibodies, but mediates directly through immunocompetent cells, i.e., T-lymphocytes. Some T-cells, called helper cells and supressor cells, modulate both the humoral and the cellular systems, chiefly by secreting chemical messengers called lymphokines, such as gamma-interferon. These biologically active factors either attack and destroy the foreign cells (i.e., T-cytotoxicity) or stimulate the macrophages to destroy the same (Young and Cohn 1988).

Several reports are available on the increased T-cell activity, when mice were fed fermented milks containing lactobacilli (Perdigon et al. 1987, 1988; Vesely et al. 1985). Pedigon et al (1987, 1988) reported increased lymphocytic activity in mice fed with 50 μg/d of viable cultures of *S. thermophilus* and *Lb. acidophilus* for 8 days. (Table 5). In another study, Pardigon et al (1994) fed *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* at the dose of $2x10^8$ cells/ml to mice for 10 consecutive days and measured small and large intestine associated lymphoid cells. They observed significant increases in IgA secreting β-cells and T-lymphocytes in the small and large intestines. Prolonged feeding with yoghurt did not produce any histological alterations in the gut, suggesting that it would do no harm, as the mucosal barrier remained intact (Perdigon et al. 1994). However, prolonged ingestion of yoghurt induced slight side effects on the liver, with a temporary increase in the serum level of the enzyme glutamic pyruvic transaminase inflammatory reaction (Perdigon 1995c).

TABLE 5. *IN VIVO* STUDIES IN EXPERIMENTAL ANIMALS INDICATING IMMUNOMODULATING EFFECT OF LACTIC ACID BACTERIA

Model	Immunomodulator	Route	Effect	References
Mice	*Lb. casei*	Intraperitoneal	Activation of peritoneal macrophages, enhanced macrophages, enhanced activity of mononuclear phagocytic system	Kato et al (1983)
Mice	*Lb. casei*	Subcutaneous	Production of circulating antibodies for *Ps. aeruginosa*, increase in production of IgM level	Saito et al (1983)
Mice	*Lb. casei*	Intravenous or intraperitoneal	Activation of natural killer cells (also caused a local transitory cellular infiltration)	Kato et al (1984)
Mice	Yoghurt	Oral	Increased content of immunoglobulins and stimulation of lymph follicles	Bourliox (1986)
Swiss albino mice	*Lb. casei* *Lb. acidophilus*	Oral	Enhanced macrophage and lymphocytic activities	Perdigon et al (1986b)
Mice	*Lb. casei*	Oral	T-cell mediated immune response to mycobacteria	Saito et al (1987)
Mice	*Lb. acidophilus* *S. thermophilus*	Oral or intraperitoneal	Immune modulation against malignant tumours through activated macrophages lymphocytes	Perdigon et al (1987)
CD1-mice	*Lb. acidophilus* *Lb. casei*	Oral	Higher phagocytic indices, no differences in blood IgG or IgM	Moineau et al (1989)
Mice	*Lb. acidophilus* *Lb. casei*	Intraperitoneal	Enchanced resistance to encephalomyocarditis virus	Mihal et al (1990)
CD1-mice	*Lb. acidophilus* *Lb. casei, B. longum*	Oral	Increased phagocytic activity of pulmonary macrophage	Moineau and Goulet (1991a)
CD1-mice	*Lb. bulgaricus* *Lb. casei, Lb. helveticus*	Oral	No significant differences in serum IgG and IgA levels	Moineau and Goulet (1991b)
Swiss albino mice	Natural yoghurt	Oral	Increased phagocytic activity, increased protective capacity against *S. typhimurium* and *E. coli*	Perdigon et al (1991a, b)
Mice	Fermented milk	Oral	Increased serum IgG level	Saucier et al (1992)
Mice	Lysate of *Lb. bulgaricus*	Oral	Increased secretion of IL-1, increased resistance to *K. pneumoniae* and *L. monocytogenes*	Popova et al (1993)
Mice	*Lb. acidophilus*	Oral	Antibody response with enhanced proliferative response of Peyer's patch cells	Takahashi et al (1993)
BALB/c mice	*Lb. acidophilus*	Oral	Increase in IgA secreting β-cells and T-lymphocytes	Perdigon et al (1994)
Malnourished mice	*Lb. casei*	Oral	Slight increase in circulating leucocytes and phagocytic activity, increased IgA producing cells	Perdigon (1995b)
Swiss albino	*Lb. acidophilus* *S. thermophilus* *Lb. bulgaricus*	Oral or intraperitoneal	2-4 fold increase in superoxide anion production by murine peritoneal macrophages	Balasubramanya et al (1995)
Chicks	*Lb. acidophilus*	Oral	Increased antibody titre, increased resistance to enteropathogenic *E. coli* infection	Patidar (1995)

Gamma-interferon is the body's most rapidly activated defence against viruses (Batish and Prajapati 1988). Some of the recent studies on immuno-regulation by intestinal flora have shown that dietary lactobacilli influence the production of gamma-interferon, which helps in control of pathogenic organisms (Plockova et al. 1990; De Simone et al. 1991). Plockova et al (1990) have reported that feeding of *Lb. acidophilus* or *Lb. casei*, increase resistance to EMC virus in mice and also increase their interferon producing property.

In a comprehensive *in vivo* experiment by De Simone et al (1989a), involving 17 human subjects of 21 to 56 years, who were challenged with yoghurt bacteria, a progressive increase in the level of serum gamma-interferon was observed. The average gamma-interferon level was 0.42 U.I./ml initially, which increased to 0.70 U.I./ml after 28 days of consumption of *Lb. bulgaricus+ S. thermophilus* at the dose of $3x10^{12}$ cells/day. An *in vitro* experiment conducted earlier by the same workers, using human peripheral blood lymphocytes, showed that the addition of small quantity of yoghurt containing live cells, increased the production of gamma-interferon by 3-4 times (De Simone 1989b). The observation is highly significant, because the heated yoghurt did not show any stimulation. However, concanavalin-A, a T-cell mitogen and yoghurt synergistically further increased the gamma-interferon level, as compared to individuals. Along with an increase in the quantity of gamma-interferon, a corresponding increase in

the number of killed K562 tumour cells by the peripheral blood lymphocytes also increased. The heat-treated yoghurt had clearly inferior immunopotentiating properties, compared to the natural yoghurt, which indicated the importance of live lactic acid bacteria. Mihal et al (1990) observed increased resistance to encephalomyocarditis virus infection in mice given intraperitoneal dose of *Lb. acidophilus* or *Lb. casei* var *casei*, 4 days prior to infection, which indicated a specific action on the immune system.

Humoral immunity: The humoral system defends the body primarily against bacteria and toxic molecules. Its weapons are antibodies or immunoglobulins, which are synthesized and secreted by ß-lymphocytes (Young and Cohn 1988). During an encounter with foreign particles (antigens), the ß-cells proliferate and some of their progeny become memory cells, which will respond to the same antigen faster the next time, while most of the progeny become plasma cells, which manufacture a large amount of the antibodies and also secrete it. The antibody binds to the antigen. Moreover, toxins are precipitated or neutralized by binding to the antibodies. It also activates the complement-cascade- system, which ultimately destroys the invading cells (Young and Çohn 1988).

The first contact with immunological defence in human involves IgG, originating in the mother and crossing the placental barrier. Subsequently milk, or more specifically the colostrum, introduces various immunological factors to infants (Plockova et al. 1990).

Conge et al (1980) were the first to observe an increase in the level of immunoglobulins, belonging to G2a class, in serum of mice fed a diet supplemented with yoghurt, containing live lactic acid bacteria. Similar results are also reported by other workers (Vesely 1985; De Simone et al. 1989a). De Simone et al (1987) reported that β-lymphocytes were found in higher percentages ($P<0.01$) in Peyer's patches from mice fed with live lactic acid bacteria after 7 and 14 days of treatment, as compared to the control group fed only milk. Human volunteers were given cultured milk, containing *Lb. acidophilus* La1 and *Bifidobacteria* over a period of 3 weeks by Link-Amster et al (1994). The volunteers were also given an attenuated *S. typhi* Ty21a, to mimic an enteropathogenic infection. They found that there was significant increase in total serum IgA and almost 4–fold increase in specific serum IgA titre against *S. typhi* Ty21a in the test group, as compared to control, which did not receive fermented milk. Further, the faecal flora analyses showed an increase in *Lb. acidophilus* and bifidobacterial count during cultured milk intake.

While monitoring humoral immunity in yoghurt fed mice, Vesely et al (1985) observed that IgG2a and IgM increased transiently on the 15th day of feeding yoghurt and heated yoghurt, to mice as compared to control group. However, on the 30th day, all the groups were at par for all the types of immunoglobulins. Significantly higher antibody level was observed in germ-free mice, when fed yoghurt containing live bacteria, as compared to those fed heated yoghurt for a period of 8 days (Wade et al. 1984). Saucier et al (1992) observed an increase in serum IgG level in weaned mice, receiving UHT milk, fermented by a mixture of 8 strains of lactic acid bacteria, as compared to those fed with bacterial suspension in unfermented milks. However, they did not observe significant differences in % phagocytosis in any of the treatments, but the mice fed fermented milk showed slightly longer survival after intranasal infection of *K. pneumoniae*. From this study, it could be revealed that fermentation is essential for the beneficial effects on the immune system, as bacteria suspended in UHT milk did not prove beneficial. Further, once the fermented milk is heat–treated, the effect goes off, which indicates the importance of live lactic acid bacteria. In an another study, when different species of lactic acid bacteria were used for feeding, only *Lb. delbrueckii* subsp. *bulgaricus* showed higher level of antibody titre (Moineau and Goulet 1991b), while others did not, probably due to strain variations. During feeding stored yoghurt, Perdigon et al (1995c) found that yoghurt stored up to 20 days could increase the anti-SRBC (sheep red blood cells) antibodies in mice, but the specific IgA against *S. typhimurium* increased only with fresh yoghurt. With respect to total immunoglobulins, the 5 day stored yoghurt showed 3–fold increase, while 15 day and 20 day stored yoghurt had no effect (Perdigon et al. 1995c).

Bacterial cell wall peptidoglycan and their N-acetyl muramyl peptide derivatives are known to have immunomodulatory effects and similar activities have been attributed to lactic acid bacteria (Perdigon et al. 1988). In this direction, Link-Amster et al (1989) examined the effect of a *Lb. bulgaricus* soluble extract on the humoral immune response to an oral enteropathogenic *E. coli* vaccine in white mice. Sera taken on 29 and 40 days were found to have 2-3 fold higher IgG titres to *E. coli* O antigen

from animals which had received a lactobacillus extract, as compared to control animals, receiving *E. coli* alone. Small intestinal lavage fluids also showed 2-3 fold higher IgA titres but low IgG titres. A patent has been granted to Link and Pahud (1991) for an immunostimulant preparation, containing N-acetyl muramyl peptide, derived from cell wall of *Lb. delbrueckii* subsp. *bulgaricus*, which has been found to promote immune response against Gram-negative enteropathogenic bacteria.

Patidar (1995) fed five groups of chicks with milk fermented by different strains of *Lb. acidophilus* for 8 days and then measured the antibody titre by haemagglutination inhibition test in sera collected upto 5 weeks after feeding. He observed that HI-titres increased up to third week and then gradually declined in all treated groups, as compared to control group fed only milk. The significant variations in the titre were observed among the strains of lactobacilli used. However, among different strains, *Lb. acidophilus* C2 showed the highest antibody titre. In another study, the same worker observed that *Lb. acidophilus* feeding offered greater protection to chicks against *E. coli* infection. In a challenge study, two groups of chicks were fed milk fermented with *Lb. acidophilus* and one group was fed milk for 16 days and all were intraperitoneally injected with pathogenic *E. coli* CH. The mortality rate in *Lb. acidophilus* C2 fed group was 27.3%, as compared to 90% in control group (Patidar 1995).

Tomioka and Saito (1992) gave a dose of 2.4-4.5x10^6 cells of different lactobacillus species to mice and after 24 h, were intraperitoneally infected with *E. coli*. The surviving mice score after 7 days was zero in untreated group, but it was highly variable with different lactobacilli. The highest 80% survival was reported in the group fed *Lb. acidophilus*. Similar type of protection against *Pseudomonas aeruginosa* and *Listeria monocytogenes* was also offered by *Lb. acidophilus* and *Lb. casei*. The action was mainly based on the activation of host macrophage by lactobacilli in either T-cell dependent or T-cell independent manner, causing a remarkable enhancement of mobilisation of blood monocytogenes to the site of infection and an elevation of antimicrobial function (Tomioka and Saito 1992).

Sharpe et al (1973) have reported that lactic acid bacteria may not always produce beneficial effects on the host. *Lb. casei* subsp. *rhamnosus* can produce endocarditis or abscesse. Some strains of *Lb. acidophilus* and *Lb. plantarum* under special conditions, may possess undesirable properties.

Tomioka and Saito (1992) from their experiments in feeding lactobacilli to mice have thought that certain substance produced by milk fermentation due to lactobacilli, such as certain casein peptide of some of the cell wall components produced by digestion of these organisms play an important role in augmentation of the host immune system. Further studies in this direction are necessary.

Antitumour properties of fermented milks

Cancer is the term associated with a variety of disorders that are characterized by the abnormal growth of cells (Keating 1985). The lactobacilli may fight against cancer by either controlling the intestinal putrefaction or retarding the activity of enzymes that convert procarcinogen to carcinogen or by activating the immune system (Keating 1985) (Table 6).

Recently, several workers have reviewed the possible anticarcinogenic effects of fermented milks containing live lactobacilli (Reddy et al. 1983; Friend and Shahani 1984; Goldin and Gorbach 1984; Keating 1985; Fernandes et al. 1990; IDF 1991; Adachi 1992). Acidophilus milk has been found to reduce the activity of faecal ß-glucosidase, ß-glucuronidase, nitroreductase and azoreductase, which are the enzymes that convert procarcinogens into carcinogens (Goldin and Gorbach 1984).

Several investigators have tried to locate the antitumour factor in fermented milks. Bogdanov et al (1975) isolated a glycopeptide fragment from the cell wall of *Lb. bulgaricus* and termed it as blastolysin. This compound exhibited antitumour activity against sarcoma-180 and Ehrlich ascites tumour. The cured animals retained permanent immunity to their respective tumours. These workers were unable to demonstrate any *in vitro* effects and concluded that this agent activated the animal's immunological mechanisms (Bogdanov et al. 1978). Ayebo et al (1981) also separated antitumour component of yoghurt by dialysis. Mice infected with Ehrlich tumour cells showed 33% reduction in tumour growth, when treated with this yoghurt dialysate for 7 days. However, in *in vitro* experiment, this antitumour effect could not be confirmed (Ayebo et al. 1982) and they concluded that this *in vivo* inhibition was through immune response. Reddy et al (1983) reported that diet supplemented with cultured yoghurt inhibited the proliferation of Ehrlich ascites tumour in mice. Similar results were also reported by other researchers (Fernandes et al. 1990; Friend et al. 1992).

TABLE 6. SELECTED REPORTS SHOWING ANTICARCINOGENIC PROPERTIES OF FERMENTED MILKS CONTAINING DIETARY LACTOBACILLI

Experimental host	Agent	Effect	References
Rats challenged with 1-2 dimethyl hydrazine (DMH)	*Lb. acidophilus*	Delayed appearance of colon tumours	Goldin and Gorbach (1980)
Mice with sarcoma 180, L 1210 leukemia and chemically induced MLA K-1 tumours	*Lb. casei*	Inhibition of tumour cell proliferation	Kato et al (1981)
Mice infected with Ehrlich ascite tumour	Yoghurt dialysate	25 to 30% reduction in tumour growth, as compared to control	Friend and Shahani (1984) Reddy et al (1983)
Mice induced with methylazoximethane	*Lb. arabinosus*	Strengthened tumoricidal effect	Iwasaki et al (1983)
Rats challenged with DMH	*Lb. acidophilus* *S. thermophilus*	Reduced mortality rate	Shackelford et al (1983)
Healthy adults	*Lb. acidophilus*	2-4 fold reduction in faecal ß- glucuronidase, azoreductase, nitroreductase	Goldin and Gorbach (1984)
Mice	*Lb. bulgaricus* *Lb. casei*	Increased activity of ß- glucuronidase and ß-glucosidase	Perdigon et al (1986a)
Mice injected with sarcoma cells	Scandinavian ropy sour milk	50-75% inhibition of sarcoma cells	Toba et al (1987)
Chickens, piglets	Yoghurt	Reduced ß-glucuronidase and ß-glucosidase activities	Cole et al (1984, 1987)
Humans with colon cancer	*Lb. acidophilus*	8 of 14 patients showed mean decrease of 43% in ß-glucuronidase activity	Lidbeck et al (1989)
Mice induced with intestinal tumour by DMH	Yoghurt	7-10 days feeding inhibited development of carcinoma	Perdigon et al (1995a)
HeLa, HEp-2 and HFs-9 cell lines	*Lb. acidophilus* *Lb. casei*	70-94% cytotoxicity against tumour cells	Manjunath (1987)
HeLa and HEp-2 cell lines	*Lb. acidophilus* *Lb. casei*	25-100% cytotoxicity against tumour against tumour cells	Sontakke (1992)
HT-29 colon cancer cell lines	*Lb. helveticus* *Lb. acidophilus* *Bifidobacteria* Yoghurt cultures	All, except *Lb. acidophilus* decreased growth rate of cancer cells	Baricult et al (1995)

Kato et al (1981) and Yokokura et al (1984) suggested that *in vivo* antitumour activity of *Lb. casei* YIT 9018 might be macrophage- dependent, because the level of tumour inhibition was found to decrease during treatment with carageenan (an antimacrophage agent). In addition, it did not show any effect in *in vitro* experiment. Perdigon et al (1986c) have also suggested that the activation of the immune system plays a role in the antitumour activity. They noticed that lactobacilli were able to activate macrophage cells in mice.

Cultured dairy products have shown antimutagenic activities on chemical and faecal mutagens (IDF 1991). Using healthy human subjects, Hosoda et al (1996) demonstrated that administration of *Lb. acidophilus* LA-22, showed remarkable decrease (71.9% on average) in faecal mutagenicity.

According to recent reports on antitumour activity of fermented milks, it will be premature to conclude its effect on human beings. However, experiments based upon provoking tumour cells on animals and *in vitro* studies on human cancer cell lines indicate the possibility that fermented milks may play an important role in our drive to flight against cancer.

Conclusion

Fermented milks are known since ages as an important diet item with a therapeutic value. These earlier beliefs of the society are now being confirmed scientifically. Every year, several research reports dealing with multifarious benefits of consuming fermented milks are pouring in, especially containing live lactobacilli. Regarding stimulation of immunity and fighting against cancer, hundreds of positive records are available, but it still needs further investigation, as the reports available so far have one or the other limitations. These are (a) Different species of experimental animals, which may not form the good models for this type of investigation, relating to humans were used. (b) It is always difficult to draw well-founded conclusions from *in vitro* experiments and (c) One of the main problems

in generalizing the results obtained is the involvement of a huge number of types and strains of microorganisms, which may be quite different from each other in their properties and effects.

Hence, there is a need to select universal strains and conduct *in vivo* trials in well defined models or actual cell lines on large scale, so as to arrive at valid conclusions.

References

Adachi S (1992) Lactic acid bacteria in the control of tumors. In: Wood BJB (ed). The lactic acid bacteria in health and disease. Elsevier Applied Science, London and New York, pp 233-261

Alm L (1991) The therapeutic effects of various cultures: An overview. In: Robinson RK (ed) Therapeutic Properties of Fermented Milks. Elsevier Applied Science, London and New York, pp 45-64

Anon (1971) The summary of reports on Yakult. The Yakult Honsha Co Ltd, No. 6-3 Chome, Nihonbashi-Honcho, Tokyo, Japan

Aranaud-Battandier F (1982) Immunologic characteristics of isolated gut mucosal lymphoid cells. In: Strober W, Hanson LA, Sell KW (eds) Recent Advances in Mucosal Immunity. Raven Press, New York, p 289

Ayebo AD, Shahani KM, Dam R (1981) Antitumour component(s) of yoghurt fractionation. J Dairy Sci 64:2318-2323

Ayebo AD, Shahani KM, Dam R, Friend BA (1982) Ion-exchange separation of the antitumour component(s) of yoghurt dialyzate. J Dairy Sci 65:2388-2390

Balasubramanya NN, Lokesh BR, Ramesh HP, Krishnakantha TP (1995) Effect of lactic microbes on superoxide anion generating ability of peritoneal macrophages and tissue histopathology of murines. Indian J Dairy Biosciences 6:28-33

Baricault L, Denariaz G, Houri JJ, Barley C, Sapin C, Trugnan G (1995) Use of HT-29, a cultured human colon cell line to study the effect of fermented milks on colon cancer cell growth and differentiation. Carcinogenesis 16:245-252

Batish VK, Prajapati JB (1988) Interferon-The potential viral killer. Everyman's Science 23:123-129

Bellanti JA, Kadlec SJ (1985) Introduction to immunology. In: Bellanti JA (ed) Immunology. WB Sandera Co, Philadelphia, pp 1-15

Bendich A (1993) Physiological role of antioxidants in the immune system. J Dairy Sci 76:2789-2794

Berg RD (1983) Translocation of indigenous bacteria from the intestinal tract. In: Hentges DJ (ed) Human Intestinal Microflora in Health and Disease. Academic Press, New York, pp 333-352

Berg RD (1992) Translocation and the indigenous gut flora. In: Fuller R (ed) Probiotics. Chapman and Hall, London, pp 55-85

Bianchi Salvadori B, Camaschella P, Cislaghi S (1988) Effect of yoghurt, lactic acid bacteria and bifidobacteria on translocation of *E. coli* in the lymph system. Int Symp Intest Microecol, Alghero

Bloksma N, Ettekoven H, Hothuis FM, Van Coorlle JL, De Renver MJ, Kreeflenmarg JG, Willers JM (1981) Effects of lactobacilli on parameters of non-specific resistance of mice. Medical Microbial Immunol 170:45-53

Blogdanov IG, Dalev PG, Gurevich AI, Kowsov MN, Malkova VP, Plemyamikova LA, Serokina IB (1975) Antitumour glycopeptides from *Lb. bulgaricus* cell well. FEBS Letters 57:259-261

Bogdanov IG, Velichokov VT, Gurevich AI (1978) Antitumour action of glycopeptides from the cell wall of *Lb. bulgaricus*. Bull Expt Biol Med 84:1750-1753

Bourlioux P (1986) Interet de lingestion volontaire de microorganisms: cas de la microflora du yaourt. Cah Nutr Diet 21:204-208

Camaschella P, Cislaghi S, Bianchi Salvadori B (1988) Proprieta probiotiche dei batteri lattici dello yoghurt e fenmeno di traslocazione batterica. Rec Progr Gastroent Nutr Padiat, Nepoli

Campos M, Godson D, Hughes H, Babiuk L (1993) The role of biological response modifiers in disease control. J Dairy Sci 76:2407-2417

Cantey JR (1978) Prevention of bacterial infections of mucosal surfaces immune secretory IgA. Adv Exp Med Biol 107:461-470

Cole CB, Fuller R (1987) The effect of dietary fat and yoghurt on colonic bacterial enzymes (ß-glucosidase and ß-glucuronidase) associated with colon cancer. Food Microbiol 4:77-81

Cole CB, Anderson PH, Philips SM, Fuller R, Hewitt D (1984) The effect of yoghurt on the growth, lactose utilizing gut organisms and ß-glucuronidase activity of caecal contents of a lactose-fed, lactase-deficient animal. Food Microbiol 1:217-222

Conge GA, Gouache P, Desormeau-Bedot JP, Loisillier F, Oemonnier D (1980) Comparative effects of live yoghurt and heated yoghurt enriched diets on the murine immuno-system. Reprod Nutr Develop 20:929-936

Dave JM, Prajapati JB (1994) Lactic acid bacteria as antibacterials against foodborne pathogens and food spoilage organisms. In: Microbes for better living. MICON-94 and 35th AMI Conference, Mysore, pp 361-367

De Simone C (1986) Microflora, yoghurt and the immune system. Int J Immunotherapy. Suppl. II:19-23

De Simone C, Bianchi Salvadori B, Negri R, Ferrazzi M, Baldinelli L, Vesely R (1986) The adjuvant effect of yoghurt on production of gamma-IFN by Con-A-stimulated human peripheral blood lymphocytes. Nutr Rep Int 33:419-433

De Simone C, Vesely R, Negri R, Bianchi Salvadori B, Tzantzoglou S, Cilli A, Lucci L (1987) Enhancement of immune response of murine Peyer's patches by a diet supplemented with yoghurt. Immunopharmacol Immunotoxicol 9:87-100

De Simone C, Bianchi Salvadori B, Jirillo E, Baldinelli L, Di Fabio S, Vesely A (1989a) Yoghurt and the immune response. In: Fermented Milks, Current Research. John Libbey Eurotext, London, pp 63-67

De Simone C, Bianchi Salvadori B, Jirillo E, Baldinelli L, Bitonti F, Vesely R (1989b) Modulation of immune activities in humans and animals by dietary lactic acid bacteria. In: Chandan RC (ed) Yoghurt: Nutritional and Health Properties. National Yoghurt Association, Virginia, USA, pp 201-213

De Simone C, Rosati E, Moretti S, Bianchi Salvadori B, Vesely R, Jirillo E (1991) Probiotics and stimulation of the immune response. Europ J Clin Nutr 45 (Suppl 2):32-34

De Simone C, Ciardi A, Grassi SL, Tzantzoglou S, Trinchieri V, Moretti S, Jirillo E (1992) Effect of *B. bifidum* and *Lb. acidophilus* on gut mucosa and peripheral blood β-lymphocytes. Immunopharmacol Immunotoxicol 14:331-340

Donawho CK, Kripke ML (1991) Evidence that the local effect of ultra violet radiation on the growth of murine melanomas is immunologically mediated. Cancer Res 51:4176

Fernandes CF, Shahani KM, Standinger W, Amer MA (1990) Anticarcinogenic properties of lactobacilli as modulated by immune factors. Brief Communication of the 23rd Int Dairy Congress, Montreal, Oct, 8-12, 1990. Belgium, Vol 1, p 178

Friend BA, Shahani KM (1984) Antitumour properties of lactobacilli and dairy products fermented by lactobacilli. J Food Prot 47:717-723

Friend BA, Farmer RE, Shahani KM (1992) Effect of feeding and intraperitoneal implantation of yoghurt culture cells on Ehrlich ascites tumor. Milchwissenschaft 37:708-710

Furukawa N, Takahashi T, Yamanata Y (1996) Effects of supernatant of Peyer's patch cell culture with kefir grain components on the mitogenic response of thymocyte and splenocyte in mice. Animal Sci Technol 67:153-159

Gandhi DN, Nambudripad VKN (1979) An introduction to different acidophilus milk products and its concentrates. Indian Dairyman 31:813-818

Garg SK (1988) *Dahi-A* fermented indigenous milk product. Indian Dairyman 40:57-60

Gilliland SE (1985) Influence of bacterial starter cultures on nutritional value of foods: Improvement of lactose digestion by consuming foods containing lactobacilli. Cult Dairy Prod J 20:28-33

Gilliland SE (1989) Acidophilus milk products: A review of potential benefits to consumers. J Dairy Sci 72:2483-2494

Gilliland SE, Nelson CR, Maxwell C (1985) Assimilation of cholesterol by *Lactobacillus acidophilus*. Appl Environ Microbiol 49:377-381

Golde DW (1991, The stem cell. Scientific American 265: 36-43

Golde DW, Gasson JC (1988) Harmones that stimulate the growth of blood cells. Scientific American 259:62-70

Goldin BR, Gorbach SL (1980) Effect of *Lb. acidophilus* dietary supplementation on 1, 2- dimethylhydrazine dihydrochloride induced intestinal cancer in rats. J Natl Cancer Inst. 64:263-265

Goldin BR, Gorbach SL (1984) The effect of milk and *Lactobacillus* feeding on human intestinal bacterial enzymes activity. Am J Clin Nutr 39:756-761

Goodwin JS, Burns EL (1991) Aging, nutrition and immune function. Clin Appl Nutr 1:85

Goulet J, Saucier L, Moineau S (1989) Stimulation of the non-specific immune response of mice by fermented milks. In: Chandan RC (ed) Yoghurt- Nutritional and Health Properties National Yoghurt Association, McLean, USA, pp 187-199

Greene WC (1993) Aids and the immune system. Scientific American (Special issue) 269:67-73

Hashimoto S, Seyama Y, Yokokura T, Mutai M (1985) Cytotoxic factor production by Kupffer cells elicited with *Lb. casei* and *Corynebacterium parvum*. Cancer Immunol Immunother 20: 117-121

Hatcher GE, Lambrecht RS (1993) Augmentation of macrophage phagocytic activity by cell-free extracts of selected lactic acid producing bacteria. J Dairy Sci 76:2485-2492

Hosoda M, Ashimoto R, He F, Morita H, Hosono A (1996) Effect of administration of milk fermented with *Lb. acidophilus* LA-2 on faecal mutagenicity and microflora in the human intestine. J Dairy Sci 79:745-749

IDF (1988) Fermented milks: Science and Technology. Bulletin of International Dairy Federation, No 227:1-137

IDF (1991) Cultured dairy products in human nutrition. Bulletin of International Dairy Federation, No 255:10-20

Iwasaki I, Yumoto N, Iwase H, Ide C (1983) Potentiation of large intestinal tumorigenicity of cycasin derivative by high fat diet and lactobacillus in germ free mice. Acta Pathol Japan, 33:1197-1204

Kandler O, Weiss N (1986) Regular, non-sporing Gram- positive rods. In: Bergey's Manual of Systematic Bacteriology, Vol 2, Wilkins and Wilkins, Baltimore and London, pp 1208-1234

Rato I, Kobayashi T, Tokokura T, Mutai M (1981) Antitumour activity of the *Lb. casei* in mice. Gann 72:517-523

Kato I, Tokokura T, Mutai M (1983) Macrophage activation by *Lb. casei* in mice. Microbiol Immunol 27:611-618

Kato I, Tokokura T, Mutai M (1984) Augmentation of mouse natural killer cell activity by *Lb. casei* and its surface antigens. Microbiol Immunol 28:209-217

Keating K (1985) The role of cultured dairy products in the prevention of stomach cancer. Cult Dairy Prod J 20:13-14

Khedekar CD, Dave JM, Sannabhadti SS (1990) Effect of feeding *acidophilus* milk on the faecal lactobacilli and coliform counts on human volunteers. Indian Dairyman 43:237-241

Kim HS (1988) Characterization of lactobacilli and bifidobacteria as applied to dietary adjuncts. Cult Dairy Prod J 23:6-9

Klupsch HJ (1983) Bioghurt- Biogarde-acidified milk products with optimum qualities. North Europ Dairy J 49:29-32

Korobkina GS, Brents MYA, Kaliniva N, Voroheva VM, Shamanova GP (1981) Development of dried *acidophilus* milk formulae for infant feeding. Voprosy Pitaniya No 1:51-54

Koroleva NS (1982) Special products (*Kefir, Koumiss*, etc.). In: Proceedings of XXI International Dairy Congress, Vol 2, Mir Publishers, Moscow, pp 146-152

Lang F (1980) Unusual cultured milk product developments. Milk Ind 82:22-24

Lang F, Lang A (1975) *Acidophilus* milk products: Little known cultured milks of great potential. Milk Ind 77:4-6

Laxminarayana H (1984) Nutritive and therapertic properties of fermented milk. Indian Dairyman 36:329-336

Lidbeck A, Gelther D, Orrhage K, Ottova L, Brismar B, Gustafssom JA, Rafter J, Nord CE (1989) Effect of *Lb. acidophilus* fermented milk on the intestinal microflora, faecal β-glucuronidase and dietary intake in colon cancer patients. In: Fermented Milks, Current Research, John Libbey Eurotext, London p 273

Link-Amster H, Hoang KY, Pahud JJ, Munoz-Box R (1989) Increase in human immune response in mice to an oral *E. coli* vaccine through a lysozyme digest of *Lb. bulguricus*. In: Fermented Milks, Current Research, John Libbey Eurotext, London, pp 275-276

Link-Amster H, Rochat, Saudan KY, Mignot O, Aeschlimann JM (1994) Modulation of specific humoral immune response and changes in intestinal flora mediated through fermented milk intake. FEMS Immunol Medical Microbiol 10:55-63

Link H, Pahud JJ (1991) Immunostimulating agent, European patent application EP 0 432-490 A2

Manjunath H (1987) Effect of lactic acid bacteria on tumor cell lines. Ph.D. Thesis, Kurukshetra University, Kurukshetra, India

Metchnikoff E (1908) The prolongation of life. G Putnam's Sons, New York

Moineau S, Goulet J (1991a) Effect of feeding fermented milk on the pulmonary macrophage activity in mice. Milchwissenschaft 46:551-554

Moineau S, Goulet J (1991b) Effect of fermented milks on humoral immune response in mice. Int Dairy J 1:231-239

Moineau S, Boutin Y, Goulet J (1989) Effect of fermented milks on the immune response in mice. J Dairy Sci 72 (Suppl 1): Abstr No 429

Mihal V, Lackovic C, Plockova M, Brezina P (1990) Protective effect of *Lb. acidophilus* and *Lb. casei* on encephalomyocarditis virus induced disease in mice. Food and Agril Immunol 2:205-209

Nader de Macias ME, Apella MC, Romeno NC, Gonzales SN, Oliver G (1992) Inhibition of *Shigella sonnei* by *Lb. casei* and *Lb. acidophilus*. J Appl Bacteriol 73:407-411

Nader de Macias ME, Romeno NC, Apella MC, Gonzales SN, Oliver G (1993) Prevention of infections produced by *E. coli* and *Listeria monocytogenes* by feeding milk fermented with lactobacilli. J Food Prot 56:401-405

Nossal GJV (1993) Life, death and the immune system. Scientific American 269:21-30

Patidar SK (1995) Effect of *Lb. acidophilus* feeding on immune response in chicks. M.Sc. Thesis, Gujarat Agricultural University, Anand, India

Paubert- Braquet M (1992) Milk products: A new approach to immuno-modulation. Biofutur 109:39-41

Paul WE (1993) Infectious diseases and the immune system. Scientific American 269:57-63

Perdigon G, Alvarez S, Nader de Macias ME, Margni RA, Oliver G, Halgadom AA (1986a) Lactobacilli administered orally induces release of enzymes from peritoneal macrophages in mice. Milchwissenschaft 41:344-348

Perdigon G, Nader de Macias ME, Alvarez S, Medici M, Oliver G, Holgado AA (1986b) Effect of a mixture of *Lb. casei* and *Lb. acidophilus* administered orally on the immune system of mice. J Food Prot 49:986-993

Perdigon G, Alvarez S, Nader de Macias ME, Margni RA, Oliver G, Holgado AA (1986c) Effect of perorally administered lactobacilli on macrophage activation in mice. Infect Immunity 53:404-410

Perdigon G, Nader de Macias ME, Alvarez S, Oliver G, Holgado AA (1987) Enhancement of immune response in mice fed with *Str. thermophilus* and *Lb. acidophilus*. J Dairy Sci 70:919-926

Perdigon G, Nader de Macias ME, Alverez S, Oliver G, Holgado AA (1988) Systemic augmentation of the immune response in mice by feeding fermented milks with *Lb. casei* and Lb. acidophilus. Immunology 63:17-23

Perdigon G, Alvarez S, Nader de Macias ME, Medici M (1989) Effect of lactic acid bacteria orally administered and of yoghurt on the immune system. In: Fermented Milks, Current Research. John Libbey Eurotext, Paris, pp 77-84

Perdigon G, Nader de Macias ME, Alvarez S, Oliver G, Holgado AA (1990) Prevention of gastrointestinal infection using immunobiological methods with milk fermented with *Lb. casei* and *Lb. acidophilus*. J Dairy Res 57:255-264

Perdigon G, Alvarez S, Holgado AA (1991a) Immuno adjuvant activity of oral *Lb casei*.: Influence of dose on the secretory immune response and protective capacity in intestinal infections. J Dairy Res 58:485-496

Perdigon G, Alvarez S, Nader de Macias ME, Savoy de Ciori G, Medici M, Nunez de Kairuz M (1991b) Behaviour of natural and heated yoghurt in the immune system and preventive capacity on enteric infections. Milchwissenschaft 46:411-416

Perdigon G, Rachid M, Budeguer MV, Valdez C (1994). Effect of yoghurt feeding on the small and large intestine associated lymphoid cells in mice. J Dairy Res 61:553-562

Perdigon G, Alvare S, Rachid M, Aguero G, Gobbato N (1995a) Immune system stimulation by probiotics. J Dairy Sci 78:1597-1606

Perdigon G, Aguero G, Alvares S, Allori G, Holgado AA (1995b) Effect of viable *Lb. casei* feeding on the immunity of the mucosal and intestinal microflora in malnourished mice, Milchwissenschaft 50:251-256

Perdigon G, Alvarez S, Medici M, Vintini E, Giori GS, Kairuz MN, Holgado AA (1995c) Effect of yoghurt with different storage periods on the immune system in mice. Milchwissenschft 50:367-371

Pereyra BS, Lemonniev D (1993) Introduction of human cytokines by bacteria used in dairy foods. Nutr Res 13:1127-1140

Plockova M, Mihal V, Lackovic V (1990) Therapeutic and immunomodulating properties of lactobacilli used in dairy industry. In: Brief Communications of the 23rd Int Dairy Congr, Montreal, Oct 8-12, 1990, Belgium, Vol 1, p 194

Papova P, Guencheva G, David Rova G, Bogdanov A, Pocelli E, Opalchenova G, Kutzarova T, Koycher C (1993) Stimulation effect of deodan (an oral preparation from *Lb. bulgaricus* Lb-51) on monocytes/macrophages. Int J Immunopharmacol 15:25-37

Prajapati JB (1995) Fundamentals of Dairy Microbiology. Akta Prakashan, Nadiad, India, pp 99-117

Prajapati JB, Shah RK, Dave JM (1987) Survival of *Lb. acidophilus* in blended spray dried *acidophilus* preparations. Aust J Dairy Technol 42:17-21

Puhan Z, Zambrini AV (1992) Overview of current availability and technology of fermented milks in IDF member countries. Bulletin of International Dairy Federation No 277:22-27

Read AE, McCarthy CF, Heaton KW, Laidlow J (1966) *Lactobacillus acidophilus* (ENPAC) in treatment of hepatic encephalopathy. Brit Med J 1:1267-1269

Reddy CV, Friend BA, Shahani KM, Farmer RE (1983) Antitumor activity of yoghurt components. J Food Protect 46:8-11

Roitt IM (1988) Essential Immunology, 6th edn. Blackwell Scientific Publications, Oxford

Saito I, Sato K, Horikawa Y (1983) Enhanced humoral antibody production and delayed hypersensitivity responses in mice by *Lb. casei*. Hiroshima J Med Sci 32:223-225

Saito H, Tomiko H, Nagashima K (1987) Protection and therapeutic efficacy of *Lb. casei* against experimental murine infections due to *Myco. fortuitum* complex. J Gen Microbiol 133:2843-2851

Sandine WE, Muralidhar KS, Elliker PR, England DC (1972) Lactic acid bacteria in food and health: A review with special reference to enteropathogenic *Escherichia coli* as well as certain enteric diseases and their treatments with antibiotics and lactobacilli. J Milk Food Technol 35:691-702

Sandine WE (1979) Role of lactobacilli in the intestinal tract. J Food Prot 42:259-262

Sato K (1984) Enhancement of host resistance against *Listeria* infection by *Lb. casei* : Role of macrophages. Infect Immun 44:445-451

Saucier L, Julien M, Cheour F, Letarte R, Goulet J (1992) Effect of feeding lactic acid bacteria and fermented milk on specific and non-specific immune responses of mice infected with *K. pneumoniae* AD-1. J Food Prot 55:595-600

Schiffrin EJ, Rochat F, Link-Amster H, Aeschilimann JM, Donnet-Huges A (1995) Immunomodulation of human blood cells following the ingestion of lactic acid bacteria. J Dairy Sci 78:491-497

Sellars RL (1991) *Acidophilus* products. In: Robinson RK (ed) Therapeutic Properties of Fermented Milks. Elsevier Applied Science, London, pp 81-115

Shah RK, Prajapati JB, Dave JM (1986) Innovations in *acidophilus* product technology. Indian Dairyman 38:24-29

Sharma N, Gandhi DN (1981) Preparation of acidophilin; I. Selection of the starter culture. Cult Dairy Prod J 16:6-10

Sharpe ME (1979) The genus *Lactobacillus*. In: Starr MP, Stolp H, Truper HG, Balows A, Schlegel HG (eds) The Prokaryotes: A handbook on habitates, isolation and identification of bacteria. Vol 11, Springer-verlag, Berlin, pp 1653-1679

Sharpe M, Hill R, Lapage S (1973) Pathogenic lactobacilli. J Med Microbiol 6:281-286

Sontakke AT (1992) Beneficial activities of selected human strains of lactobacilli in milk for its use as dietary adjunct. Ph.D. Thesis, Gujarat Agricultural University, Anand, India

Shackelford LA, Rao DR, Chawan CB, Pulusani SR (1983) Effect of feeding fermented milk on the incidence of chemically induced colon tumours in rats. Nutr Cancer 5:159-164

Takahashi T, Oka T, Iwana H, Kuwata T, Yamamato Y (1993) Immune response of orally administered lactic acid bacteria. Biosci Biotechnol Biochem 57:1557-1560

Tamime AY, Robinson RK (1985) Yoghurt-Science and Technology. Permagon Press Ltd, Oxford, UK

Toba T, Kitazawa H, Itoh T, Adachi S, Kumano N (1987) Antitumor activity of ropy sour milk. FEMS Microbiol Rev 46: p 76 (F 10)

Tomioka H, Saito R (1992) Lactic acid bacteria in the support of immuno compromised hosts. In: Wood BJB (ed) The Lactic Acid Bacteria, Vol 1, Elsevier Applied Science, London, pp 263-296

Vesely R, Negri R, Bianchi Salvadori B, Lavezzari D, De Simone C (1985) Influence of a diet addition with yoghurt on the mouse immune system. EOS J Immunol Immunopharmacol 51:30-35

Visek WJ (1990) Diet and cancer. In: Prinsley DM, Sandstead HH (eds) Nutrition and Aging. Alan R Liss Inc, New York, p 301

Wade S, Corthiewr G, Morean C, Besnier MD (1984) L'ingestion de yaourt vivant modificelle la response immunitaire? Bulletin of International Dairy Federation, No 179, Poster I

Yokokura T, Kato I, Matsuzaki T, Mutai M, Satoh N (1984) Antitumor activity of *Lb. casei* YIT 9018-effect of administration route. Jap J Cancer Chemothera 11:2427-2433

Yokokura T, Nomoto K, Shimizu T, Nomoto K (1988) Enhancement of hematopoietic response of mice by subcutaneous administration of *Lb. casei*. Intect Immunity 52:156-160

Young JD, Cohn ZA (1988) How killer cells kill. Scientific American 258:38-44

Yuguchi R, Goto T, Okonogi S (1992) Fermented milks, lactic drinks and intestinal microflora. In: Nakazava Y, Hosono A (eds) Functions of Fermented Milks, Challenges for the Health Sciences. Elsevier Applied Science, London and New York, pp 247-273

Received 5 August 1996; revised 22 November 1996; accepted 1 December 1996

Effect of Additives on the Stability of Mango Aroma Concentrate During Storage

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The storage behaviour of aroma concentrate from 'Alphonso' mango was studied at room temperature (26-28°C), 2°C and -18°C in the presence and absence of certain additives viz., glucose + glucose oxidase + catalase system, sodium sulphite and ascorbic acid. It was noticed that aroma constituents underwent changes during two months storage at room temperature, leading to the loss of typical aroma profile in the product without additives. Carbonyls, alcohols and oxygenated terpenes showed lower values during storage. Even in the presence of additives, the stability was extended only by a month. In contrast to this, aroma concentrates stored at 2°C, were stable for a period of 6 months without additives and upto 10 months with additives. At -18°C, they were stable for 12 months even without additives. The results were substantiated by the evaluation of ready-to-serve beverages prepared from stripped juice concentrates incorporated with 100% level of the respective stored aroma samples.

Keywords: Mango aroma concentrate, Additives, Glucose, Glucose oxidase, Catalase, Sodium sulphite, Ascorbic acid, Sensory evaluation.

Developments in the methods of aroma recovery have led to the production of new aroma or essence solutions, which possess much of their characteristic flavour. It has been shown that the aroma concentrates, after reconstitution with stripped juice concentrates, were similar to 'cut-back' juice concentrates. However, it is advantageous to store the aroma concentrates and juice concentrates separately so that more and more aroma concentrate could be used instead of 'cut-back' juice (Bomben et al. 1968; Mannheim et al. 1967). The aroma concentrate can also find applications in flavouring various dairy products like ice creams, yoghurt, milk shakes, etc (Sulc 1984). The stability of these aroma concentrates becomes an important factor during storage, since there is always a time gap between their preparation, until they are used for the manufacture of final product for which they are intended. Various studies on the stability of apple aroma (Kruzer and Shafer 1983 ; Guadagni et al. 1967) orange essence (Guadagni et al. 1970) and banana aroma (Khalil 1990) have been reported.

One of the main reasons for the deterioration of aroma concentrates is their sensitivity to oxygen and light. The presence of heavy metal ions may also bring about the changes in the aroma solutions (Sulc 1984). Therefore, studies were undertaken on the stability of tropical fruit aroma concentrate of 'Alphonso' mango, using certain antioxidants like glucose + glucose oxidase + catalase system, sodium sulphite and ascorbic acid in order to exclude the oxygen present in them and store the product in dark.

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Materials and Methods

Raw materials : Fully ripe 'Alphonso' mangoes with uniformly developed colour and flavour with firm texture purchased from a local market were washed under running tap water. The stem portion/ any surface black specks were removed and pulped in APV pulper (Aluminium Plant and Vessels Co. Ltd, England) fitted with 0.6 mm dia sieve.

Aroma recovery : Aroma from mango was recovered in a pilot plant model aroma recovery unit (Holstein and Kappert Gmbh, 4600, Dortmund, Germany) with the feed rate of 400 $1h^{-1}$ by adopting the principles of evaporation and fractional distillation. Percent evaporation of water from pulp was 40.

Concentration of aroma stripped juice : The aroma stripped juice was concentrated two folds in a forced circulation evaporator (120 kg water evaporation h^{-1}, fabricated at CFTRI workshop as per CFTRI design) operating at 45°C.

Preparation of samples of aroma concentrates: The aroma concentrates of mango obtained in the aroma recovery unit were used. The aroma solutions (50 ml) with each of the following additives : a) glucose (10 mg) + glucose oxidase (2 mg) + catalase (2 mg), b) ascorbic acid (5 mg), c) sodium sulphite (10 mg) were sealed in glass tubes separately under nitrogen and stored at 26-28°C, 2°C and -18°C. For each storage temperature, aroma solution without any additives served as control.

Chemical analysis : The aroma distillates were analyzed for ester (Leonard and Willard 1960), carbonyls (Lappan and Clark 1951), alcohols (Snell

et al. 1953) and total oxygenated terpenes (Latrasse et al. 1982).

Measurement of dissolved oxygen : The dissolved oxygen content was determined by an oxygen analyser model PM 520, (Spinco, Madras) with an electrode consisting of silver anode and a potassium chloride paste/gold cathode.

Evaluation of beverages : Ready-to-Serve beverages were prepared from stripped juice concentrates having 15% pulp, 16° Brix and 0.25% acidity. The aroma concentrates were incorporated in the beverages at 100% level. Beverages were evaluated by the scientific staff of the department.

Results and Discussion

Dissolved oxygen : The relationship of dissolved oxygen with respect to different additives at different temperatures are presented in Fig 1, which shows that the oxygen content was reduced to a very great extent over a period of 3 months at room temperature (Fig 1a) with respect to the additives tried. However, sodium sulphite was found to be more effective than other additives. Both glucose + glucose oxidase + catalase and ascorbic acid systems were found to have equal effect, but to a lesser extent than sodium sulphite. At 2°C (Fig 1b) and -18°C (Fig 1c), a gradual decrease in the oxygen content was observed over a period of 12 months, when additives were used. Among them, sodium sulphite was found to bring down the oxygen level to about 0.5 ppm at both the temperatures. Other additives were found to be less effective.

Changes in volatile components : Fig 2, 3, 4 and 5 depict the effect of additives on the stability of aroma solution in terms of different groups of volatiles like esters, carbonyls, alcohol and oxygenated terpenes at different temperatures, respectively (26-28°C, 2°C, -18°C). An increase in ester content (Fig 2a) and decrease in carbonyls (Fig. 3a), alcohols (Fig. 4a) and oxygenated terpenes (Fig. 5a) was observed in aroma solution stored at room temperature without any additives. This means that aroma solutions undergo changes at a much faster rate at room temperature without any additives. However, when additives were used, no significant changes in terms of different groups of components were noticed. Sodium sulphite was found to be the most effective in retaining the aroma strength as compared to glucose + glucose oxidase + catalase and ascorbic acid systems. These changes in chemical groups of aroma solution in the absence of additives (reducing substances causing reducing atmosphere) are likely to be due to oxidation. These oxidative changes adversely affected the organoleptic quality of aroma concentrate (Table 1).

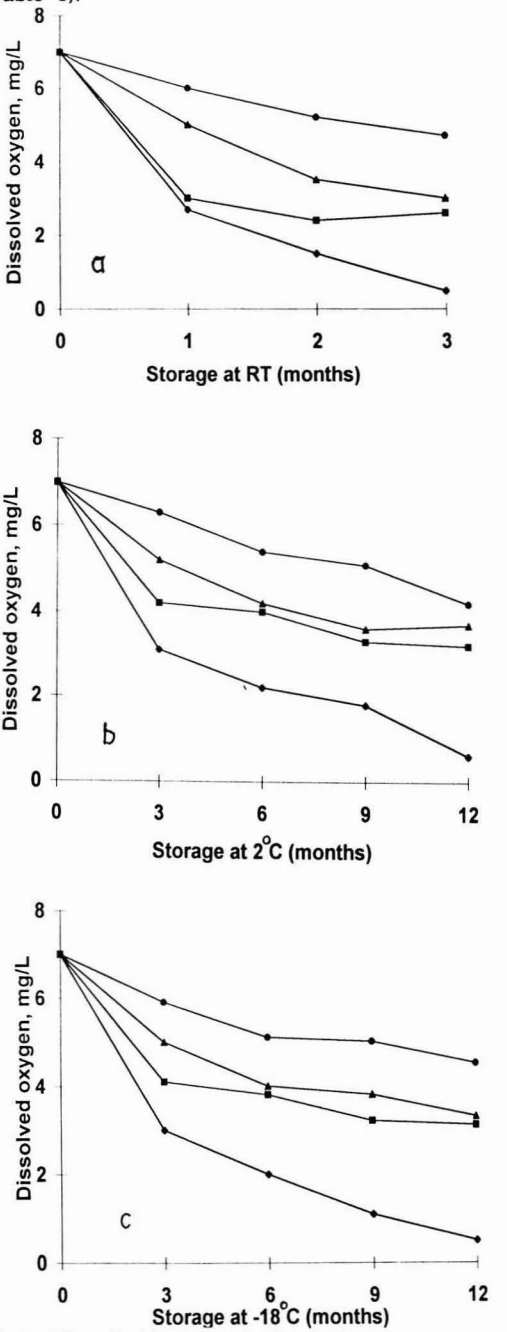

Fig 1. Effect of additives on the dissolved oxygen content of aroma concentrate during storage at different temperatures
a) 26-28°C b) 2°C c) -18°C
● control, ■ ascorbic acid, ▲ glucose + glucose oxidase + catalase, ◆ sodium sulphite

Eventhough, the additives were found to be quite effective in bringing down the oxygen level in the aroma solutions, the storage at room temperature was limited to only 2-3 months. After 3 months storage at room temperature, the aroma solutions were found to be contaminated by fungal growth. Hence, it is apparent that storage at room temperature is not satisfactory.

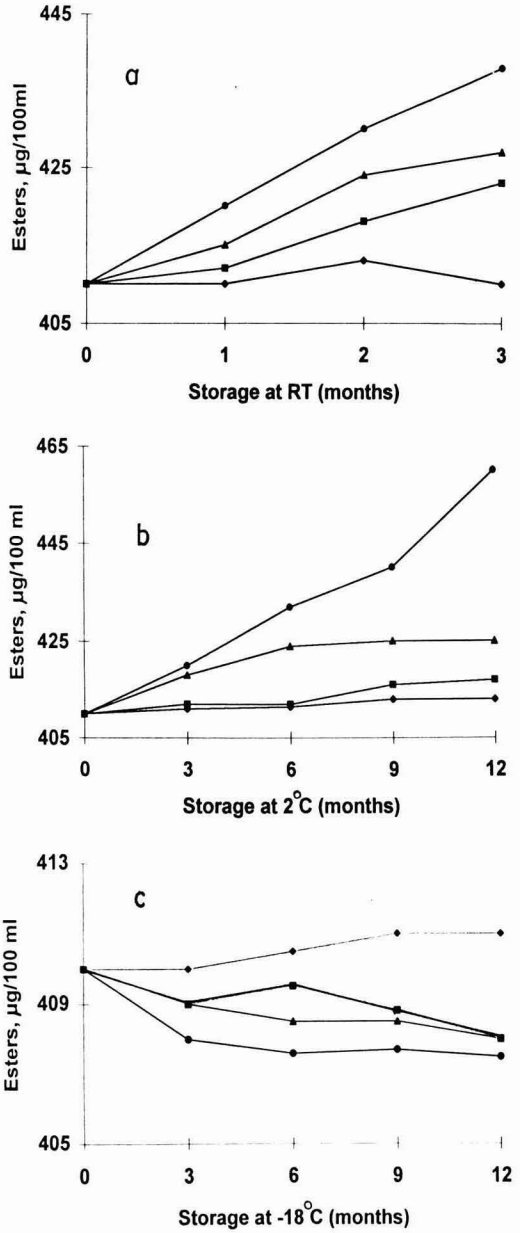

Fig 2. Effect of additives on the volatile ester content of aroma concentrate during storage at different temperatures
a) 26-28°C b) 2°C c) -18°C
● control, ■ ascorbic acid, ▲ glucose + glucose oxidase + catalase, ◆ sodium sulphite

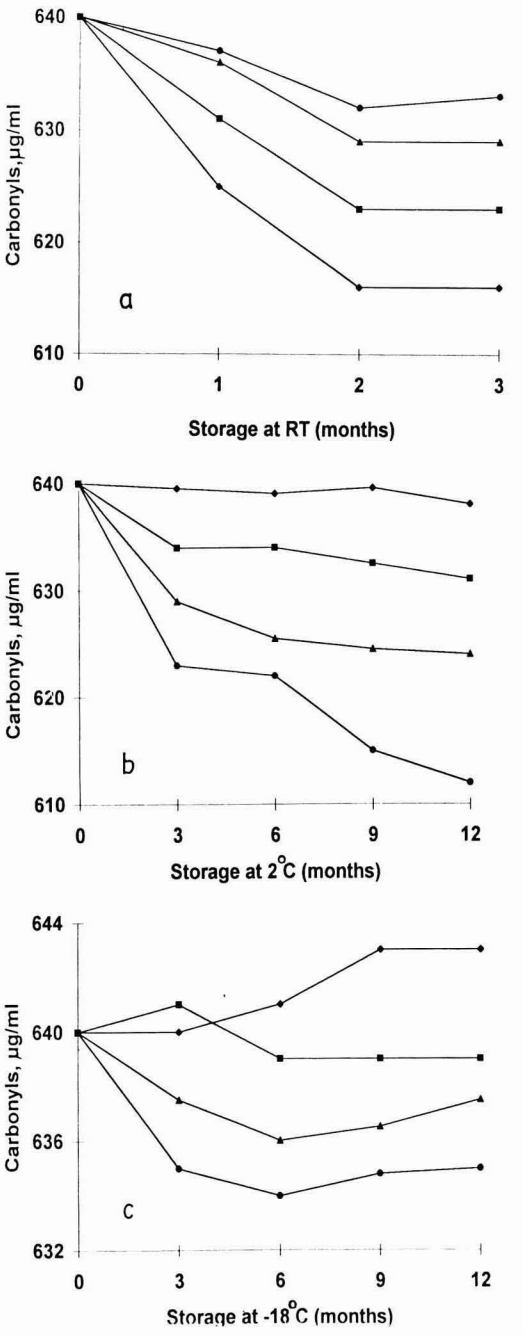

Fig 3. Effect of additives on the volatile carbonyl content of aroma concentrate during storage at different temperatures
a) 26-28°C b) 2°C c) -18°C
● control, ■ ascorbic acid, ▲ glucose + glucose oxidase + catalase, ◆ sodium sulphite

The relationships of different groups of volatiles like esters (Fig 2b), carbonyls (Fig 3b), alcohols (Fig 4b) and oxygenated terpenes (Fig 5b), with respect to different additives on stability of aroma solution at 2°C are presented. Fig 2b shows that without additives, there was an increase in ester content over a storage period of 12 months. However, the

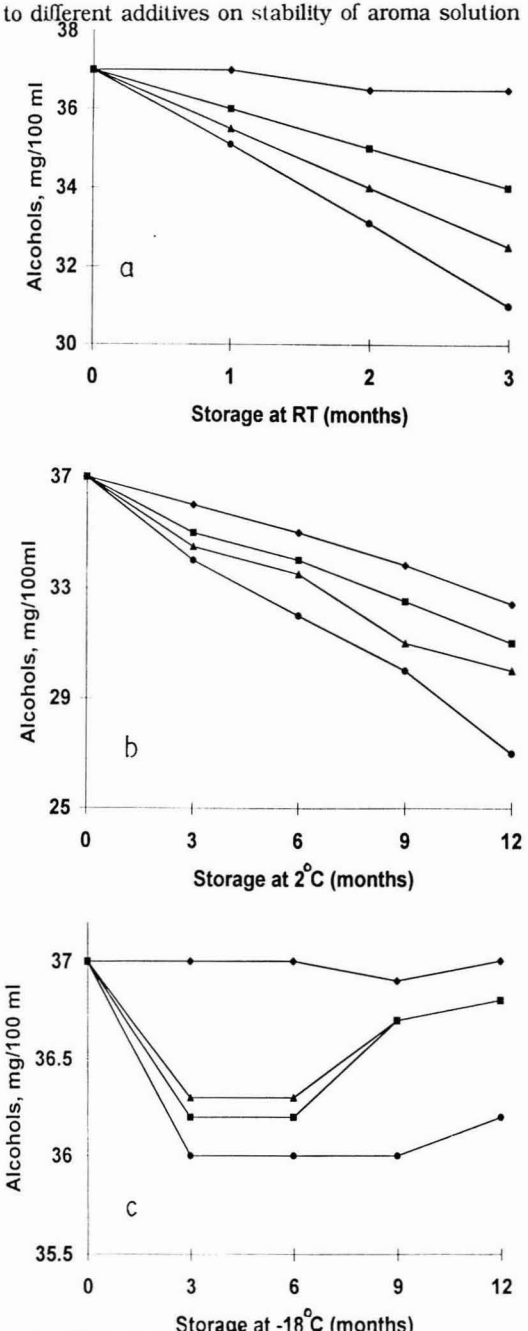

Fig 4. Effect of additives on the volatile alcohol content of aroma concentrate during storage at different temperatures
a) 26–28°C b) 2°C c) -18°C
● control, ■ ascorbic acid, ▲ glucose + glucose oxidase + catalase, ◆ sodium sulphite

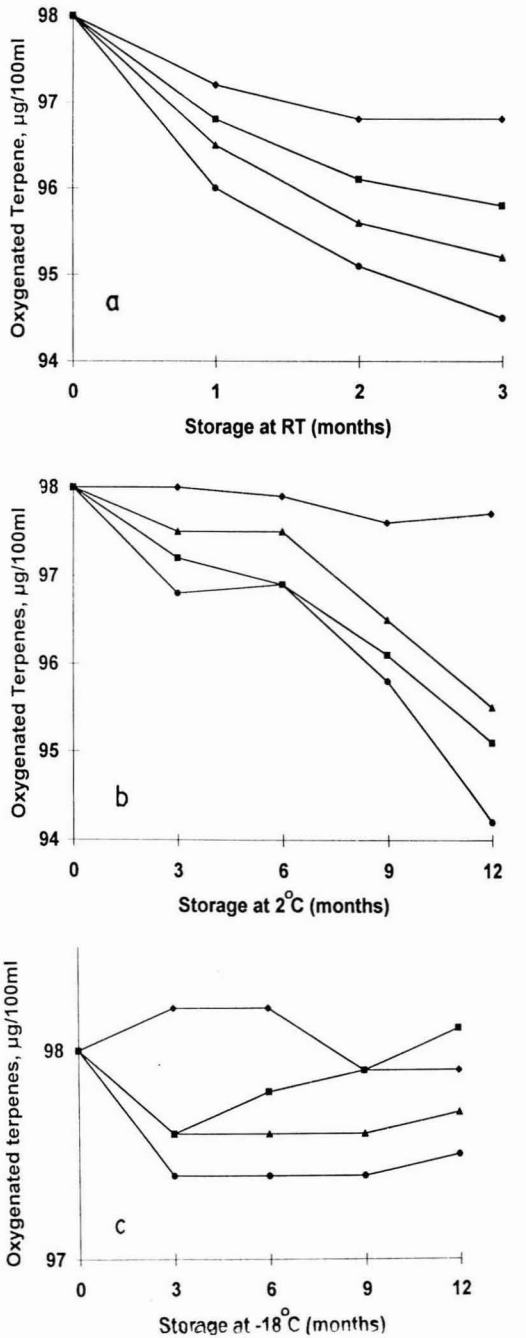

Fig 5. Effect of additives on the oxygenated terpene of aroma concentrate during storage at different temperatures
a) 26–28°C b) 2°C c) -18°C
● control, ■ ascorbic acid, ▲ glucose + glucose oxidase + catalase, ◆ sodium sulphite

TABLE 1. SENSORY PARAMETERS FOR THE EVALUATION OF AROMA CONCENTRATE DURING STORAGE AT DIFFERENT TEMPERATURES

	Storage temperature	RT				2°C				–18°C
Treatment	Storage period, months	1	2	3	4	6	9	10	11	12
Control		TM	SO	OF + FG	FG	TM	OF	OF	OF	TM
Sodium sulphite		TM	TM	TM	FG	TM	TM	TM	OF	TM
Glucose + glucose oxidase + catalase		TM	TM	SO	FG	TM	TM	SO	OF	TM
Ascorbic acid		TM	TM	SO	FG	TM	TM	SO	OF	TM

TM - Typical mango, SO - Slightly overripe, OF - Off flavour, FG - Fungal growth

increase was not significant upto 6 months of storage. Incorporation of additives in the aroma solutions was found to inhibit changes in ester content. No significant changes were observed upto 10 months storage, when sodium sulphite was used. Other additives like glucose + glucose oxidase + catalase and ascorbic acid were found to have similar effect, but to a lesser extent than sodium sulphite.

Similarly, no significant differences in the aroma solutions with respect to carbonyls (Fig 3b), alcohols (Fig 4b) and oxygenated terpenes (Fig 5b) were observed, when additives were used. Here again, sodium sulphite was found to be more effective than the other two additives. Although there was a gradual decrease in the groups of components, when used without additives, it was not found to be significant upto 6 months of storage. Increase in ester content could due to the gradual esterification of alcohols with volatile acids present in the aroma solution. Changes in carbonyls can be attributed to oxidation by dissolved oxygen, which on addition of additives (reducing agents), is preferentially taken up.

At –18°C, volatile fractions like esters (Fig 2c), carbonyls (Fig 3c), alcohols (Fig 4c) and oxygenated terpenes (Fig 5c) did not show any significant change, when used with or without additives upto 12 months of storage.

The studies indicate that it is possible to extend the shelf life of aroma concentrate by the use of antioxidants. The most effective antioxidant was found to be sodium sulphite, which reduced the oxygen content to below 0.5 ppm. These aroma concentrates can be used for flavouring various dairy products like ice cream, yoghurt, milk shakes etc.

References

Bomben JL, Guadagni DG, Harris JG (1968) Stability of single strength orange juice made with aroma solution or cut back. Food Technol 22: 230-232

Guadagni DG, Okano S, Harris JG (1967) Effect of temperature on storage stability of apple essence obtained from different varieties. Food Technol 21: 665-668

Guadagni DG, Bomben JL, Mannheim HC (1970) Effect of temperature on stability of orange aroma solution. J Food Sci 35: 279-281

Khalil KE (1990) Studies on preparation of fruit juice concentrates based on Bangalore blue grapes and banana, Ph.D Thesis, University of Mysore, India

Kruzer G, Schafer I (1983) Untersuchungs methoden and beweetungskriterien fur naturliche apfel aromakonzentrate. Lebensm Industrie 30: 13-18

Lappan GR, Clark LC (1951) Colorimetric method for determination of traces of carbonyl compounds. Anal Chem 23: 541-542

Latrasse A, Lantin B, Mussilon P, Sarris J (1982) Aroma quality of raspberry I. Rapid colorimetric determination of an aroma index using vanillin in concentrated sulphuric acid. Lebensm Wiss Technol 15: 19-21

Leonard RM, Willard BR (1960) Changes in volatile constituents during baking of sherry wine by Tressler process. Food Technol 14: 30-33

Mannheim HC, Bomben JL, Guadagni DG, Morgan Jr AJ (1967) Evaluation of orange aroma solutions obtained by a new vacuum stripping method. Food Technol 21: 469-473

Snell FD, Snell CT, Snell CA (1953) Primary and secondary alcohols. Colorimetric methods of analysis. Van Nostrands, Princeton, NJ. p 39

Sulc D (1984) Fruit juice concentration and aroma separation. Confructa 28: 258-318

Received 12 September 1995; revised 6 January 1997; accepted 7 January 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 3, 200-203

Storage Performance of Kinnow Mandarins in Evaporative Cool Chamber and Ambient Condition

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Freshly harvested 'Kinnow' fruits were subjected to treatments of bavistin, semperfresh (sucrose polyester wax) and their combinations. These fruits and an untreated lot (as control) were kept at room temperature (16.75 to 20.61°C and 62-95% RH) and in a big size evaporative cool chamber (14.56 to 18.69°C and 84-96% RH). Results indicated that fruits treated with bavistin in combination with semperfresh could be kept upto 40 days in cool chamber as against 15 days at room temperature. Data on shelf life, physiological loss in weight (PLW), spoilage and retention of vitamin C indicate that the cool chamber is an ideal on-farm storage technology for maintaining proper fruit quality and market acceptability of kinnow.

Keywords: Kinnow, Storage, Cool chamber, Ambient, Quality, Vitamin C.

'Kinnow' mandarin is the first generation hybrid between the 'Willow leaf mandarin' *(Citrus deliciosa)* and 'King orange' *(Citrus nobilis)* (Ghosh 1985). They are grown in Punjab, Haryana, Himachal Pradesh and Rajasthan. They are also produced around Coorg (Sandhu et al. 1983; Srivastava and Bopiah 1978). It is gaining popularity in North India mainly due to its wide adaptability, high yield and attractive fruit colour. Recently, attempts have been made for the export of this important fruit to the neighbouring countries. Owing to lack of information on appropriate post-harvest treatments and on-farm storage, the fruits not only lose their prime quality, but also encounter a substantial post-harvest loss.

The excellent quality fruits are generally available for only one or two months i.e., from January to mid February in North India. Although the ambient temperature prevailing during this period of the year is low, the ambient RH remains still very low. This results in severe desiccation of fruits within a very short period after harvest, if not marketed immediately. Unfortunately, due to improper post-harvest infrastructure facilities, most of the time, the fruits are not transported immediately after harvest and are held back in the farm, resulting in considerable quality deterioration.

On-farm storage plays a vital role in maintaining quality soon after harvest. Survey of literature shows that some amount of work has been done on various post-harvest aspects of 'Kinnow'. Sinha (1987) reported that the 'Kinnow' mandarins could be kept in cool chamber up to 60 days. Mann (1978) reported that 'Kinnow' fruits could be stored for 3 months at 36-40°F with 85-90% RH, when packed in perforated polyethylene bags. A combination of fungicide and wax emulsion was found to be effective in loss reduction of 'Kinnow' (Singh et al. 1988). Dhutt et al (1991) established the efficacy of HDPE film on retention of quality of 'Kinnow' fruits. Kumar et al (1990, 1991) studied the effects of different storage conditions on the shelf life of 'Kinnow'. The existing information does not provide substantial evidence regarding its on-farm storage.

Therefore, an on-farm storage study was conducted with the objective to retain post-harvest quality and extend the shelf life of 'Kinnow' mandarins, using a big size evaporative cool chamber developed at IARI, New Delhi (Roy 1984), which maintained high humidity and relatively low temperature.

Materials and Methods

Freshly harvested 'Kinnow' fruits from the Punjab were brought to the PHT-laboratory of the Division of Fruits and Horticultural Technology, IARI, New Delhi within 48 h of harvest in the month of February. The fruits were sorted out for elimination of bruised, punctured and damaged ones. Soon after sorting, the fruits were treated with (i) 500 ppm of bavistin, (ii) semperfresh - a sucrose polyester wax (1.5%), and (iii) mixture of bavistin (500 ppm) and semperfresh (1.5%). One lot of fruits was kept as untreated control. These were then stored (i) at room temperature (16.75 to 20.61°C with 62 to 95% RH) and (ii) in large cool chamber (14.56 to 18.69°C with 84 to 96% RH) in perforated plastic crates. The cool chamber was developed at IARI having hollow cavity walls filled with fine riverbed sands with drip system of watering device from the top periphery for bringing down the

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temperature by evaporative cooling. The inner size of the chamber was 1728 cubic feet having 12' x 12' x 12, which could store about 8 metric tonnes of fresh fruits and vegetables. The above treatments were replicated 4 times and each replication consisted of 25 kg of fruits. After periodical observations on various quality parameters were made, the data were analyzed using ANOVA technique in a randomized block design (Panse and Sukhatme 1978).

Physiological loss in weight : The physiological loss in weight was determined by periodical weighing of fruits and the differential weight loss was expressed in % with respect to storage time and pre-treatments.

Spoilage : The visible symptoms of rotting/ spoilage were recorded at periodical intervals. The cumulative % of rotting occurred with respect to advancement in storage period was calculated and data were presented in graphical form.

Shelf life : The shelf life of fruits was determined by judging the unmarketability parameters viz., shrivelling and softening, which were mainly due to physiological loss in weight (PLW). Ten percent physiological loss in weight was considered as an index of end point of shelf life of fruits.

Vitamin C : The vitamin C content of the juice was estimated by visual titration method with 2, 6 dichlorophenol indophenol dye (Anon 1966).

Peel texture : The textural property of peel was determined with the help of Instron texture measuring device (model 4201). A 1.5 mm probe was used to puncture the peel, using 100 N load cell having a crosshead speed of 100 mm per min. The force required to puncture the peel i.e., "peak break" was recorded and the values were expressed in 'Newton'.

Results and Discussion

It is evident from the data presented in Fig.1 that there was a sharp increase in PLW of fruits stored at room temperature, whereas the increase in PLW was found to be very slow in fruits stored in cool chamber. Semperfresh treatment resulted in checking the PLW under both the storage conditions. However, the prominent difference between the control and the waxed fruits was noticed only from the 10th day onwards (Fig. 1). Therefore, the effect of semperfresh in restricting the PLW upto 10% level (marketable) was found to be 15 days and 41 days at room temperature and in cool chamber, respectively. However, the

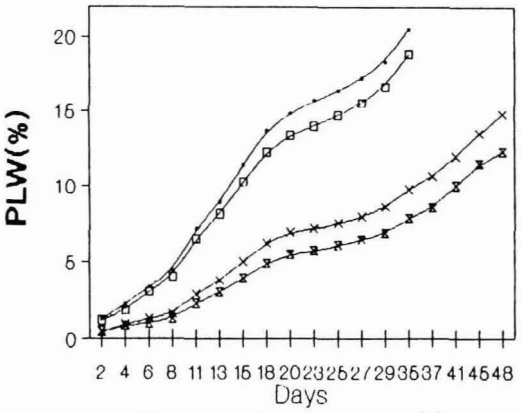

Fig 1. Effect of fungicide and waxing on PLW of kinnow at room temperature (RT) and in big size cool chamber (CC)
● RT Control, ⊟ RT SF+Bav., ✕ CC Control, ⧖ CC SF+Bav., SF - Semperfresh, Bav. - Bavistin

untreated fruits became unmarketable (10% PLW) on the 13th and 35th day at room temperature and in cool chamber, respectively. Semperfresh acted as a barrier for loss of moisture from the fruit surface and this was found to be more effective in high RH and relatively low temperature condition, prevailing in the cool chamber as compared to room temperature. Singh et al (1988) made similar observations on the effect of fungicides and wax emulsions, when stored under ambient condition.

Data presented in Fig 2 and 3 clearly indicate that there was significant control of rotting by combined application of semperfresh with bavistin under both the storage conditions. First visible symptoms of spoilage were observed on the 18th

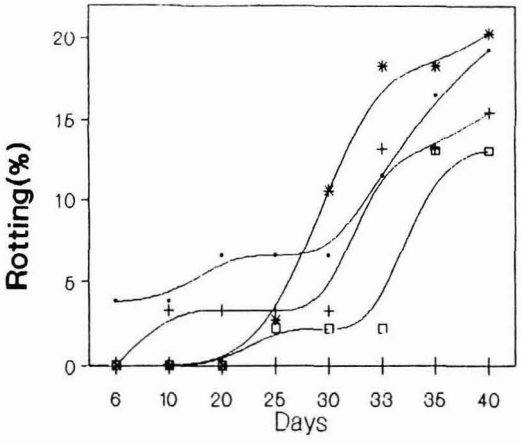

Fig 2. Effect of fungicide and waxing on spoilage of kinnow at room temperature
● RT Control, -+- RT Bav, -✳- RT SF, ⊟ RT SF+Bav, SF - Semperfresh, Bav. - Bavistin

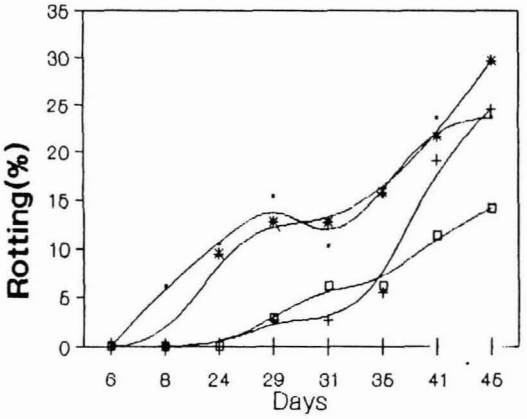

Fig 3. Effect of fungicide and waxing on spoilage of Kinnow in big size cool chamber (CC)
-●- CC Control, -+- CC Bav, -✳- CC SF,⊟ CC SF+Bav, SF - Semperfresh, Bav - Bavistin

and 24th day of storage at room temperature and in cool chamber, respectively. However, the untreated (control) fruits started showing visible symptoms of fungal infection even within the first week of its storage under both the conditions. The delay in appearance of symptoms of decay causing organism in the combination treatment with semperfresh and bavistin is primarily due to delay in senescence and prophylactic action of bavistin. High RH coupled with relatively low temperature in cool chamber further delayed the senescence, which resulted in less spoilage as compared to the room temperature storage. However, during the later part of the storage, the rotting in the cool chamber-stored fruits showed an increasing trend possibly due to onset of senescence followed by fungal infection at high humidity conditions.

Retention of vitamin C was found to be better in fruits stored in cool chamber as compared to those stored at room temperature. It went on decreasing with the advancement of storage time irrespective to pretreatment and storage conditions. The differential content of vitamin C among the treatments was found to be non-significant at room temperature. However, semperfresh-treated fruits showed significantly high content of vitamin C in cool chamber upto 15 days of storage. At the end of storage in cool chamber (40 days), the differences in vitamin C % among the treatments became non-significant (Table 1). The decrease in ascorbic acid content during storage was also reported by Chundawat et al (1978).

It is evident from the data presented in Table 1 that there were significant differences in

TABLE 1. EFFECT OF WAXING AND FUNGICIDE APPLICATION ON SHELF-LIFE, RETENTION OF VITAMIN C AND PEEL TEXTURE OF 'KINNOW' MANDARINS DURING STORAGE

Attribute	Treatments				
	Control	Bavistin	Semper-fresh	Semper-fresh + bavistin	C.D. at 5%
Shelf life in days					
i) RT	13	13	15	15	-
ii) CC	25	37	30	40	-
Vitamin C (mg/100ml)					
i) RT 15th day	15.20	14.81	15.16	15.16	NS
ii) RT 40th day	10.23	9.48	8.98	8.46	2.02
iii) CC 15th day	16.38	15.36	21.68	22.52	2.55
iv) CC 40th day	14.84	16.38	16.38	16.38	2.15
Texture of peel (Newton)					
i) 0 day	3.50	3.60	3.50	3.50	N.S.
ii) 25th day at RT	7.27	7.29	4.75	6.16	0.99
iii) 25th day in CC	4.19	4.19	3.95	4.05	0.95

RT - Room temperature; CC- Cool chamber; NS - Non-significant

the peel texture among the treatments and between the storage conditions. High value of Instron reading indicated that greater force was required to puncture the peel, which became leathery on desiccation, whereas, low value signified more freshness of the peel. Therefore, it is obvious from the results presented in Table 1 that semperfresh had contributed significantly towards the maintenance of peel freshness by delaying senescence of fruits under both the storage conditions. However, this delay was found to be significant in fruits stored in the cool chamber, when compared with those stored at room temperature.

The present study clearly indicates that the evaporative cool chamber developed by IARI for the on-farm storage of fruits could significantly contribute towards the retention of post-harvest quality upto a substantial period of 40 days after harvest, if a proper fungicidal wax treatment is given prior to storage. This could help in a big way for the domestic as well as export marketing of this fruit.

Acknowledgement

The authors gratefully acknowledge Mr. S.C. Singhal, Executive Director (Hort.) and Mr. M.S. Tiwari, Manager (Hort.) of National Agricultural Cooperative Markeing Federation, New Delhi for supplying fruits for the experiment.

References

Anon (1966) Methods of Vitamin Assay. 3rd edn. Association of Vitamin Chemists, Interscience Publishers, New York, p 287

Chundawat BS, Gupta AK, Singh BP (1978) Storage behaviour of different grades of 'kinnow' mandarin fruits. Punjab Hort J 18(3/4):156–160

Dhutt AS, Randhawa JJ, Singh SW (1991) Effect of individual seal packaging in high density polyethylene (HDPE) film on storage life and quality of mandarin fruit. J Plant Sci Res 7(1/4):84–85

Ghosh SP (1985) In: Bose TK (ed) Fruits of India – Tropical and Subtropical, Naya Prakash, pp 162–218

Kumar J, Sharma RK, Singh Ran Godara RK (1990) Increased shelf life of mandarins *(Citrus reticulata)* by different storage conditions and chemicals. Ind J Agril Sci 60(2): 151–54

Kumar J, Sharma RK, Singh Ran, Godara RK (1991) Effect of modified atmosphere storage on shelf life of 'kinnow' mandarin fruit. Haryana J Hort Sci 20(3–4): 156–160

Mann SS (1978) Harvesting and storage of kinnow fruit. Punjab Hort J18: (3/4) 154–155

Panse VG, Sukhatme PV (1978) Statistical Methods for Agricultural Workers, ICAR, New Delhi

Roy SK (1984) Post-harvest storage of fruits and vegetables in a specially designed build-in space. In: Proceedings of International Workshop on Energy Conservation in Buildings. Central Building Research Institute (India), Roorkee, pp 190–193

Sandhu SS, Dhillon BS, Singh S (1983) Post-harvest application of GA and wrappers on the storage behaviour of 'kinnow'. Indian Food Packer 37(3): 65–71

Singh K, Mann SS, Chander Mohan (1988) Effect of fungicides and wax emulsion on storage of 'kinnow' mandarins at ambient conditions. Haryana J Hort Sci 117(1/2): 14–19

Sinha J (1987) Post-harvest studies on three varieties of mandarin orange *(Citrus reticulata* Blanco), Ph.D. Thesis, Indian Agricultural Research Institute, New Delhi, India

Srivastava KC, Bopiah MG (1978) Prospects of growing 'kinnow' mandarins in the southern states. Punjab Hort J 18: 139–141

Received 20 June 1995; revised 9 January 1997; accepted 13 January 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 3, 204-207

Short-chain Organic Acids in Aged Blanco Cheese

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Low molecular weight organic acids present in aged Blanco cheese were determined by gas chromatography and verified by gas chromatography-mass spectrometry. The acid profile in mg% in decreasing order was : 730 lactic, 45 oxalic, 27 butyric, 19 caproic, 14 succinic and 5 propionic. Variation coefficients for the concentration of the acids ranged between 10.41 and 31.89%.

Keywords: Organic acids, Hard cheese, Aged Blanco cheese, Gas chromatography, GC-MS.

Aged Blanco cheese is mainly manufactured in Zulia State (western state of Venezuela), by aging fresh Blanco cheese for approximately 3 to 4 months. The milk for the cheese is from dairy-type crossbred cows. The main breeds are 'Holstein', 'Zebu', 'Brown Swiss' and a native breed "Mosaico Perijanero". The milk is coagulated with rennet at 35-37°C. Subsequently, the gel is finely cut to separate the whey and the curd. The firm curd is then cut into cubes of 3 cm each, which are salted in saturated brine for 15-30 min. The salted cubes are placed in 10 to 18 kg cheese moulds and pressed for several hours. Cheeses are removed from the moulds, their surface covered with a mixture of spent coffee pulp or annato powder and aged for 3 to 4 months at room temperature (35-38°C). Aged cheeses usually have 32 to 38% moisture content, 0.8 to 1.0% acid (w/w, expressed as lactic acid), 6 to 8% sodium chloride and 28 to 30% fat. Aged Blanco cheese consumption has increased significantly in Venezuela in recent years. Its acceptance can be attributed to its strong, desirable flavour as well as its compatibility with many foods, mainly starchy foods. It is also the most salty cheese made in the region.

Little information is available on the organic acid composition of cheeses produced in Venezuela. Ferrer and Granados (1992) reported the short-chain organic acids of a fresh-type Venezuelan cheese called Palmita-type cheese. The most abundant acids in the cheese were lactic, propionic, acetic and succinic. However, this type of cheese has composition and organoleptic properties, such as flavour and texture, that are different from aged Blanco cheese.

Low molecular weight acids produced by certain lactic cultures have been correlated with flavour characteristics in cheeses such as Cheddar, Swiss, Emmental, Tilsit and Edam (Langsrud and Reinbold 1973), each of which possesses a characteristic profile due to the aging process. The organic acids arise not only from glycolysis and lipolysis, but from amino acids and certain chemical reactions (Jensen et al. 1975; Green and Manning 1982; El-Gendy et al. 1983). Some cheeses, like Latin-American cheese (Kosikowski 1982), require the direct addition of acidulants (Bevilacqua and Califano 1989). Organic acids are believed to contribute to the flavour of most aged cheeses in the world (Adda et al. 1982). They also help to understand the metabolism of microorganisms present in milk products.

The objective of this work was to determine and quantify by gas chromatography the relative concentration of volatile and non-volatile short-chain organic acids present in aged Venezuelan Blanco cheese, to characterize the acid profile that determines the important organoleptic characteristics of this cheese.

Materials and Methods

Sampling of aged Blanco cheese : Eight samples of 500 g each produced by different cottage factories of the Zulia State, were purchased from a single retailer, dealing with aged Blanco cheese. The samples were taken to the laboratory under refrigeration and immediately subjected to extraction of organic acids.

Preparation of the samples for acid extraction: A 40 g specimen was taken from each sample of cheese (by duplicate) and homogenized at high speed for 5 min with 60 ml of distilled water, to produce a 40% (w/w) emulsion, which was filtered through a Whatman No. 4 paper. The filtrate was centrifuged to separate insoluble matter from the liquid portion. This portion was subjected to extraction of organic acids.

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Volatile and non-volatile acids : Extraction and separation of organic acids by gas chromatography was performed according to the method of Lombard and Dowell (1982). Acetic, propionic, butyric, isobutyric, valeric and caproic acids were extracted with diethyl ether. The non-volatile lactic, succinic, oxalic, fumaric and malonic acids were methylated and the methyl esters were extracted with chloroform. Organic acids were identified by retention time and gas chromatography-mass spectrometry.

Determination of acid concentrations and identification : A 3300 Varian GC with an FID detector and 2m x 1/8 in stainless steel columns was connected to a 4400 Varian integrator. The columns were packed with 15% FFAP in 80/100 Chromosorb W/WA (Supelco) for the analysis of volatile acids and with 15% OV-275 in 80/100 Chromosorb W/WA (Supelco) for the analysis of non-volatile acids. Nitrogen (30 ml/min) was used as the carrier gas and air (300 ml/min) and hydrogen (30 ml/min) were used as the flammable gas mix. The injection port, column and detector temperatures and the injection volume for the analysis of volatile acids were 200, 160 and 250°C and 1.5 µl, respectively, whereas the injection port, column and detector temperatures and the injection volume for the analysis of non-volatile acids were 180, 110 and 200°C and 10 µl, respectively. Standard solutions of acids (Aldrich) were prepared at 0.1, 0.5 and 1.0 mM for each acid. The concentration of the acids was estimated by calculating the area of the peak of each acid and using the external standard method with a multilevel calibration for each acid. The concentration was expressed as mg of acid per 100 g of cheese (mg%). The GC-MS analysis was performed in a 3400 Varian gas chromatograph connected to a Finnigan Mat mass spectrometer detector, loaded with a Magnum data system software. The chromatograph was fitted with a Nukol (Supelco) 30 m x 0.25 mm capillary column for volatile acids and a DB-1701 (Supelco) 30 m x 0.25 mm capillary column for non-volatile acids. Helium was used as carrier gas at 20 cm/s. The oven was held at 100°C for 5 min, then raised to 250 at 10°C/min for volatile acids. The oven temperature was maintained at 120°C for non-volatile acids. The injector was set at 250°C in both cases. One µl of sample (prepared as described above) was injected into the chromatograph. The mass spectrometer was operated in the positive ion electron impact ionization mode with an electron energy of 70 eV.

Recovery : Tests were made for each acid, adding a known volume of standard acid solution to a sample of homogenized cheese. A sample of the same cheese was prepared without addition of the standard solution and both samples were subjected to organic acid separation and extraction process and to the chromatographic analysis.

Results and Discussion

Acid peaks were identified by comparing their retention time with those of a standard acid solution and were confirmed by GC-MS. GC retention times for acids are given in Table 1. Resolution in both the GC determination and in GC-MS determination was very good.

Results of the acid recovery study are given in Table 2. Quantitative recovery was obtained for acetic, propionic, isobutyric, butyric, valeric, caproic, lactic, oxalic, malonic, fumaric and succinic acids. Recovery was high for both volatile and non-volatile acids, except for oxalic acid, for which the recovery was low (73.3%). Similar recoveries have been reported by Biede and Hammond (1979), Harvey et al (1981) and Ferrer and Granados (1992).

Fig. 1 shows the frequency of the acids in the cheese samples. It is apparent that lactic, succinic, caproic, oxalic and propionic acids are typical of aged Blanco cheese. Lactic and succinic acids were found in all the samples. Propionic acid was also present in all the samples, but 37.5% of the samples had only traces of this acid. Butyric, caproic and oxalic acids were detected in 85% of the samples. Isobutyric acid and fumaric acid were detected in one sample. Valeric and isovaleric acids were detected only in trace amounts (<5 mg%).

In Venezuela, the short-chain organic acids were reported for Palmita-type cheese, with lactic acid (140 mg%), propionic acid, succinic acid and acetic acid as the most abundant acids (Ferrer and Granados 1992). In Cheddar cheese, acetic and propionic acids have been found in concentrations of 70 and 180 mg%, respectively (Patton 1963; Marsili 1985), whereas the concentrations of lactic

TABLE 1. RETENTION TIMES OF ORGANIC ACIDS

Volatile acids	RT, min[1]	Non-volatile acids	RT, min
Acetic	1.95	Lactic	2.61
Proprionic	2.67	Oxalic	5.19
Isobutyric	2.97	Malonic	6.30
Butyric	3.74	Fumaric	6.93
Isovaleric	4.39	Succinic	10.00
Valeric	5.69		
Caproic	8.62		

[1]RT : Retention time

TABLE 2. RECOVERY OF ORGANIC ACIDS ADDED TO AGED WHITE CHEESE

Acid	Measured X, mg%	Endogenous SD	Amount added, mg	Calculated total, X, mg%	Measured total, X, mg%	Measured total, SD	Recovery, %
Volatile							
Acetic	15.5	0.1645	52.5	68.0	64.1	0.6782	94.3
Propionic	9.5	0.0276	178.2	167.7	187.6	3.1086	100.0
Isobutyric	2.1	0.0821	256.5	258.6	239.0	1.7213	92.4
Butyric	72.2	3.3292	172.8	245.0	255.4	2.8865	104.2
Isovaleric	1.4	0.0285	184.0	185.4	186.0	8.4016	100.3
Valeric	1.2	0.0391	188.0	189.2	180.5	15.4319	95.4
Caproic	30.1	1.5383	74.4	104.5	101.9	1.4079	97.5
Non-volatile							
Lactic	2278.1	71.9519	156.0	2434.1	2525.0	69.6019	103.7
Oxalic	100.5	5.8866	23.0	123.5	90.5	5.1915	73.3
Malonic	3.7	0.0148	313.8	317.5	325.8	18.8823	102.6
Fumaric	8.1	0.0427	314.8	322.9	321.9	20.7218	99.7
Succinic	42.9	1.7197	303.3	346.2	360.6	21.3831	104.2

acid and succinic acid have been reported as 1,300 mg% and 6.4 mg%, respectively (Harvey et al. 1981). Older Cheddar cheeses (>48 days) usually have higher levels of lactic acid (Bouzas et al. 1991) ranging from 2,000 to 2,800 mg%.

Table 3 presents values of the absolute and relative acid concentrations of the short-chain organic acids present in aged Blanco cheese and they were different from the concentrations reported for other cheeses. The acids in decreasing order of concentration were : lactic, oxalic, butyric, caproic, succinic and propionic acids. As in most cheeses, lactic acid is the major acid (86.9% of the acids), but aged Blanco cheese is less acidic and has less lactic acid than aged cheeses such as Cheddar, Swiss, Emmental and Parmesan (Harvey et al. 1981; Bouzas et al. 1991). In Palmita-type cheese (Ferrer and Granados 1992), the source of acids appears to be related to glycolysis and citrate metabolism, since it is a fresh cheese. Montoya and Ferrer (1989) reported some lipolysis in Palmita-type cheese, based on the total amount of free fatty acids present in the cheese. However, butyric and caproic acids were not detected. In this study, the production of these two acids clearly indicated the lipolysis of milk fat. Their concentrations were higher than in ripened cheeses such as Swiss cheese and Emmental cheese, but lower than in Italian cheeses like Provolone and Romano, while being similar to Parmesan cheese (Woo and Lindsay 1982; Ha and Lindsay 1990). Nevertheless, the high concentrations of long-chain fatty acids indicate a significant lipolysis in Parmesan cheese (Woo and Lindsay 1982). Succinic acid is a by-product of lactate and citrate metabolism. It has been reported that it may be produced by lactobacilli from citrate (Kaneuchi et al. 1988) and by some *Enterobacter*

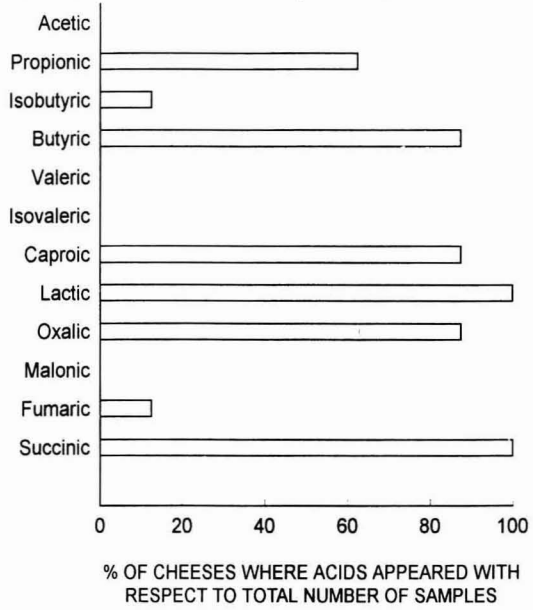

Fig 1. Incidence of low molecular weight organic acids in aged Blanco cheese samples

TABLE 3. MEAN, STANDARD DEVIATION, COEFFICIENT OF VARIATION AND RELATIVE CONCENTRATION OF ACIDS IN AGED BLANCO CHEESE SAMPLES

	Propionic	Butyric	Caproic	Lactic	Oxalic	Succinic
$\bar{X}$[1]	5.00	27.00	19.00	730.00	45.00	14.00
SD	1.57	4.86	2.67	79.58	5.55	1.46
CV%	31.89	17.83	13.81	10.94	12.34	10.41
Relative concentration, %	0.60	3.21	2.26	86.90	5.36	1.67

[1] Concentrations are average of all the samples where the acid appeared. Concentrations were not corrected by recovery factor

strains from either lactose or citrate (Urdaneta et al. 1995). These microorganisms are abundant in Venezuelan Blanco cheeses and their initial concentrations in the cheese might be 10^9 cfu/g of cheese (Ferrer et al. 1987). The concentration of propionic acid is very low in aged Blanco cheese, but as an extremely volatile acid, it can have an important role in flavour. Propionic acid may be produced by *Enterobacter* strains isolated from Venezuelan fresh Blanco cheeses from citrate (0.65% ammonium citrate) at concentrations up to 16 mg of acid/100ml of culture media (Urdaneta et al. 1995). However, the same strain has failed to produce propionic acid in skim milk. Acetic acid does not seem to be important to the flavour of this cheese, since it was found in trace amounts, although most endogenous bateria like enterococci and coliform bacteria found in fresh Blanco cheeses manufactured in Zulia State produce high quantities of acetic acid in skim milk (up to 120 mg/100ml of skim milk). Acetic acid is an important component of the flavour of Palmita-type cheese (Ferrer and Granados 1992), even though its concentration is low (7 mg%). Data presented in Table 3 also show, contrary to expectations, that aged Blanco cheese is quite homogeneous, considering that cheese samples were obtained from different manufacturers and that the age of the cheeses were also likely to be different. The coefficients of variation were small, except for propionic acid, but even 31.89% is acceptable for an acid present at low concentrates.

Since the flavour of aged Blanco cheese is very strong, it is very likely that carbonylic compounds are present. Future research will be directed towards the analysis of cheese for this class of compounds.

Acknowledgements

Support for this work was provided by Laboratorio de Alimentos, DEBS and CONDES (Institutional Financial Aid in 1991) of the University of Zulia, Maracaibo, Venezuela. The authors thank Dr. R.L. Richter (Dairy Science Laboratory, Texas A & M University, USA) for help in revising the manuscript.

References

Adda J, Gripon JC, Vassal L (1982) The chemistry of flavour and texture generation in cheese. Food Chem 9: 115-117

Bevilaequa AE, Califano AN (1989) Determination of organic acids in dairy products by high performance liquid chromatography. J Food Sci 54: 1076-1077, 1079

Bouzas J Kant CA, Bodyfelt F, Torres JA (1991) Simultaneous determination of sugar and organic acids in Cheddar cheese by high performance liquid chromatography. J Food Sci 56: 276-278

Biede SL, Hammond EG (1979) Swiss cheese flavour. I: Chemical analysis. J Dairy Sci 62: 227-237

El-Gendy SM, Abdel-Galil H, Shahim Y, Hegazi FZ (1983) Acetoin and diacetyl production by homo-and heterofermentative lactic acid bacteria. J Food Prot 46: 420-425

Ferrer A, Granados A (1992) Organic acids of low molecules in Palmita-type cheese. Food Chem 45: 311-317

Ferrer A, Urdaneta D, Rincon Z (1987) Evaluation fisico-quimica y microbiologica del queso tipo Palmita Venezolano. Rev Ciencias (Univ Zulia) 4: 133-147

Green ML, Manning DJ (1982) Development of textures and flavour in cheese and other fermented products. J Dairy Res 49: 737-748

Ha JK, Lindsay RC (1990) Method for the quantitative analysis of volatile free and total branched-chain fatty acids in cheese and milk fat. J Dairy Sci 73: 1988-1999

Harvey CD, Janness R, Morris HA (1981) Gas chromatography quantitation of sugars and non-volatile water soluble organic acids in commercial Cheddar cheese. J Dairy Sci 64: 1648-1654

Jensen JP, Reinbold GW, Washam CJ, Vedamuthu ER (1975) Role of enterococci in Cheddar cheese: Free fatty acid appearance and citric acid utilization. J Milk Technol 38: 78-83

Kaneuchi Ch, Seki M, Komagata K (1988) Production of succinic acid, citric acid and related acids by lactobacilli strains. Appl Environ Microbiol 54: 3053-3056

Kosikowski FV (1982) Cheese and fermented milk foods. 2nd edn. FV Kosikowski Publisher. Broo..'ondale, NY

Langsrud T, Reinbold G (1973) Flavour development and microbiology of Swiss cheese. A review. J Milk Food Technol 36: 593-609

Lombard G, Dowell V (1982) Procedures for gas-liquid chromatography analysis of volatile and non-volatile acid products of bacteria. CDC Laboratory Manual, Georgia

Marsili R (1985) Monitoring chemical changes in Cheddar cheese during aging by high performance liquid chromatography techniques. J Dairy Sci 62: 3155-3161

Montoya C, Ferrer A (1989) Transformaciones producidas por bacterias durante la manufactura del queso tipo Palmita. Rev Tec Ing (Univ Zulia) 12: 69-74

Patton S (1963) Volatile acids and the aroma of Cheddar cheese. J Dairy Sci 46: 856-858

Urdaneta D, Raffe D, Ferrer A, Sulbaran-de-Ferrer B, Perez M (1995) Short-chain organic acids product on glucose, lactose and citrate media by *Enterococus faecalis, Lactobacillus casei* and *Enterobacter aerogenes strains.* Bioresource Technol (In press)

Woo AH, Lindsay RC (1982) Concentration of fatty acids and flavour development in Italian cheese varieties. J Dairy Sci 67: 960-968

Received 25 May 1995; revised 15 January 1997; accepted 30 January 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 3, 208-211

Effect of Trypsin Inhibitor on Protein Quality of Black-Soybean and Mothbean Meals

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Proteins of black-soybean *(Glycin max* var *'kalitur')* and mothbean *(Vigna aconitifolius)* were found to be nutritionally poorer with protein efficiency ratio (PER) values of 0.55 and 0.90, respectively, as compared to 2.66 for casein under similar conditions. Mild processing methods viz., buffer extraction and chemical acetylation in cold to free the meals of their trypsin inhibitory activities, improved the PER values to 1.54 and 1.12. Raw defatted meals fed to rats produced extensive changes in the weight and the enzyme activities of the pancreas and small intestine. They increased the activities of protease and trypsin of pancreas and small intestine, but lowered that of amylase over the control group fed on casein. Feeding of buffer-extracted and acetylated meals, however, did not affect the activities of these enzymes, indicating the involvement of trypsin inhibitors present in defatted meals. The acid and alkaline phosphatase activities of liver and small intestine were also higher in all groups compared to control and therefore, may not be linked to the presence of trypsin inhibitors. The changes produced by the defatted soybean meal were relatively greater than the defatted mothbean meal, thereby indicating a relationship between the level of trypsin inhibitor and the protein quality.

Keywords: Trypsin inhibitor, Protein quality, Soybean meal, Mothbean meal, Protein efficiency ratio.

Black-soybean *(Glycin max* var *'kalitur')* is an indigenous variety of soybean, well established in the central India and generally consumed by tribals of the area. Mothbean *(Vigna aconitifolius)* is another indigenous crop of western Rajasthan. Raw soybean flour and other legumes have been found to retard the growth of experimental animals and protease inhibitors have been reported to be the cause of growth depression (Kakade et al. 1973; Liener and Kakade 1980). Protease inhibitors not only cause growth depression, but also hypertrophy and hyperplasia of pancreas in rats and chicks, when fed directly or as raw meal (Liener and Kakade 1980; Furukawa et al. 1987; Melmed et al. 1976; Ge and Morgan 1993). Studies on the effect of trypsin inhibitor have shown that it causes increases in pancreatic and intestinal weights (Ge and Morgan 1993), increase in pancreatic volume (Furukawa et al. 1987) and stimulation of secretion of all pancreatic enzymes by ingestion of raw soybean (Nitsan and Liener 1976). Numerous attempts were made to inactivate the protease inhibitors of soybean in order to improve its quality by autoclaving, heating and steaming (Liener and Kakade 1980), extruding (Zhang et al. 1993) and formaldehyde treatment (Nitsan and Bruckental 1977). These treatments inactivated the protease inhibitors with the improvement in the quality of protein as well as reduction of pancreatic hypertrophy in chicks and rats. Acetylation of ovomucoid, a trypsin inhibitor, resulted in the loss of its trypsin inhibitory activity (Simlot and Feeney 1966). Mothbean has also been reported to contain trypsin inhibitor, which is inactivated upon heating (Subbulakshmi et al. 1976; Mehta and Simlot 1982). The present paper discusses the effect of raw and mildly processed black-soybean and mothbean meals on the growth of rats and their organs.

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Materials and Methods

Preparation of processed meals : Black-soybean *(Glycin max* var *'kalitur')* and mothbean *(Vigna aconitifolius)* were purchased from the local market. After decortication, the meals were defatted by repeated extraction with hexane. The defatted meals were further processed either to remove trypsin inhibitor or to inactivate it by the following treatments.

Extraction with buffer : Defatted meal (100 g) was extracted with 300 ml of 0.2 M acetate buffer (pH 4.0) in cold for 10 h. Residue was re-extracted two times with 200 ml buffer, two times with distilled water and dried at 37°C (Mehta and Simlot 1982).

Acetylation : To defatted meal (50g), suspended in 500 ml of half saturated sodium acetate solution in ice-both, 5 ml of acetic anhydried was added in 5 equal increments over one hour. Residue was washed two times with minimum distilled water to remove the excess reagent and dried at 37°C (Simlot and Feeney 1966).

Animals : Forty two albino rats (25–50 g) were equalised as closely as possible for weight and kept on basal diet (Lee et al. 1979) along with mineral and vitamin mixtures for 3 days before starting on the experimental diet. The processed and raw meals were fed at 10% protein level and the amount of starch was correspondingly adjusted. After termination of the experiment (3 weeks), the rats were starved for 15 h, anaesthesized with chloroform and sacrificed. Pancreas, liver and small intestine were removed and freed from extraneous tissue.

Analytical methods : Tissues from pancreas (100 mg), liver (100 mg) or small intestine (150 mg) were homogenized in distilled water, centrifuged and the clear supernatants were used for analysis. Protein content in the homogenate was estimated by the method of Lowry et al (1951) and in the meals by micro-Kjeldahl method by multiplying with factor 6.25 (AOAC 1980). Trypsin inhibitory activity in the meals was measured with casein as substrate for the trypsin (Mehta and Simlot 1982).

The activities of amylase (Bernfeld 1955), acid and alkaline phosphatases expressed in King–Armstrong Units (KAU) (Carr 1953), trypsin with benzoyl arginine ethyl ester (BAEE) as substrate and protease with casein as substrate (Mehta and Simlot 1982) were measured after proper dilution of the homogenate. Trypsin and protease assays were done after prior incubation of the homogenate with enterokinase (0.5 mg) to activate the trypsinogen.

Results and Discussion

Defatted meals of black-soybean and mothbean contain trypsin inhibitor with activities of 49.35 µg trypsin inhibited/mg meal and 2.96 µg/mg and protein content of 47% and 25%, respectively. The meals were made free of the trypsin inhibitor either by extraction with buffer or by chemical acetylation (Table 1). These methods were milder in treatment, as they were carried out in cold as against harsher treatments like heating, autoclaving (Liener and Kakade 1980) and extrusion (Ramamani et al. 1996) normally employed for inactivation of trypsin inhibitor in soybean meal. Protein efficiency ratio (PER), which is an indicator of the protein quality, was very low for the defatted soybean meal (0.55) and for the defatted mothbean meal (0.90), when compared with the standard protein, casein (2.66) under similar conditions (Table 1). Buffer-extracted and acetylated meals gave higher PER values than the defatted meals, indicating that the removal of trypsin inhibitor accomplished the improvement in protein quality and the growth of animals. Such

TABLE 1. TRYPSIN INHIBITORY ACTIVITY AND PROTEIN EFFICIENCY RATIO OF PROCESSED MEALS OF BLACK-SOYBEAN AND MOTHBEAN

Feed	Trypsin inhibited, µg/mg meal	Protein, %	Weight gained in 3 weeks, g	PER
Casein (control)	–	–	45.90 ±4.4	2.66 ±0.16
Black-Soybean meals				
Defatted	49.35	47	6.99 ±0.28[a]	0.55 +0.05[a]
Buffer-extracted	Negligible	38	20.43 ±0.85[ab]	1.54 ±0.08[ab]
Acetylated	Negligible	41	13.62 ±0.44[ab]	1.04 ±0.11[ab]
Mothbean meals				
Defatted	2.96	25.0	9.16 ±0.63[a]	0.90 ±0.04[a]
Buffer-extracted	Nil	18.5	13.56 ±0.62[ab]	1.12 ±0.04[ab]
Acetylated	Nil	20.0	11.80 ±0.87[ab]	0.98 ±0.06[a]

[a] significant difference over control group, [b] significant difference over the corresponding defatted meal group. Values are mean ± SEM. PER – Protein Efficiency Ratio

an improvement in PER and the growth of animals over raw soybean feeding has also been reported, after it was processed by heating (Rackis et al. 1975), autoclaving (Ramamani et al. 1996; Chohan et al. 1993; Liener and Kakade 1980), chemical treatment with formaldehyde (Nitsan and Bruckental 1977) and in broad beans by autoclaving (Marquardt et al. 1976) with the resultant loss of inhibitory activity. Protease inhibitors present in soybean were also responsible for the hypertrophy and hyperplasia of pancreas of rats fed raw meal (Liener and Kakade 1980). Defatted black-soybean and mothbean meals fed to rats increased the weight of pancreas from 524mg/100g BW of the control group to 703 and 581, respectively (Table 2). The weight of small intestine had also increased for the soybean meal group, but not for the mothbean meal group. The liver weight, on the contrary, decreased in soybean meal group and without any change in mothbean group. Feeding of soybean trypsin inhibitor had shown increases in pancreatic weight (Lee et al. 1991) and pancreatic volume along with hypertrophy of acinar cells (Furukawa et al. 1987; Ge and Morgan 1993). Trypsin inhibitor also caused increases in both villus and crypt thickness in small intestine, thus increasing its weight (Ge and Morgan 1993). Feeding of buffer-extracted and acetylated soybean meals did reduce the pancreatic weight over defatted meal group, but they were still significantly higher than the control group. The weights of small intestine and liver of the processed meal groups were not different from the control group.

Measurement of enzyme activities in any tissue

TABLE 2. EFFECT OF FEEDING PROCESSED SOYBEAN AND MOTHBEAN MEALS ON THE PANCREAS, LIVER AND SMALL INTESTINE OF RATS

	Control (casein)	Soybean meal			Mothbean meal		
		Defatted	Buffer-extracted	Acetylated	Defatted	Buffer-extracted	Acetylated
Pancreas							
Weight, mg/100gBW	524.0 ±13.8	703.0 ± 8.0[a]	587.0 ±12.2[ab]	617.0 ±19.5[ab]	581.0 ±18.1[a*]	506.0 ±12.6	552.0 ±16.1
Protein, %	14.4 ± 0.5	15.9 ± 0.5[a]	14.6 ± 0.7	13.4 ± 0.8[b]	15.5 ± 1.0[a]	14.3 ± 1.3	15.2 ± 0.7
Amylase, U/mg	7.3 ± 0.4	5.3 ± 0.4[a]	7.1 ± 0.5[b]	7.0 ± 0.5[b]	5.6 ± 0.5[a]	7.1 ± 0.4	6.5 ± 0.4
Protease, U/g	90.0 ± 8.9	113.0 ± 5.6[a*]	83.0 ±10.3[b*]	68.0 ± 7.2[b]	87.0 ± 9.0	88.0 ±10.5	76.0 ± 8.1
Trypsin, U/mg	27.6 ± 2.1	41.5 ± 3.2[a]	30.2 ± 2.0[b]	27.9 ± 2.1[b]	29.5 ± 1.5	30.2 ± 1.7	30.0 ± 1.6
Liver							
Weight, g/100gBW	3.9 ± 0.2	3.1 ± 0.3[a*]	3.1 ± 0.2[a*]	3.8 ± 0.4	3.3 ± 0.3	4.0 ± 0.25	3.5 ± 0.4
Protein, %	14.1 ± 0.9	12.0 ± 0.8	13.1 ± 1.4	11.3 ± 1.2	13.5 ± 1.2	15.0 ± 0.75	14.0 ± 1.1
Alkaline phosphatase, U/g	31.3 ± 1.9	44.6 ± 1.9[a]	24.1 ± 1.2[ab]	50.1 ± 3.6[a]	35.2 ± 2.1	40.4 ± 1.3[ab*]	41.2 ± 1.0[ab]
Acid phosphatase, U/g	250.0 ±10.9	350.0 ±23.8[a]	288.0 ±14.2[a*b*]	276.0 ±18.1[b*]	311.0 ±13.8[a]	291.0 ± 9.3[a]	301.0 ±12.3[a]
Small intestine							
Weight, g/100gBW	1.8 ± 0.2	3.1 ± 0.2[a]	2.3 ± 0.2[b]	2.4 ± 0.2[a*b]	2.1 ± 0.3	1.7 ± 0.1	2.0 ± 0.2
Protein, %	3.2 ± 0.2	4.1 ± 0.2	3.6 ± 0.2	3.8 ± 0.3	3.8 ± 0.2	4.2 ± 0.2	3.8 ± 0.3
Amylase, U/g	620.0 ±59.8	476.0 ±44.0[a*]	523.0 ±49.2	464.0 ±41.7[a*]	421.0 ±40.0[a*]	460.0 ±43.1[a*]	450.0 ±29.7[a*]
Protease,	10.1 ± 0.9	15.2 ± 1.5[a]	9.6 ± 1.3[b]	8.0 ± 0.6[a*b]	10.0 ± 1.1	12.2 ± 1.0	10.3 ± 1.0
Trypsin, U/mg	1.5 ± 0.2	2.4 ± 0.3[a]	1.8 ± 0.2	1.8 ± 0.2	2.5 ± 0.2[a]	2.4 ± 0.2[a]	2.0 ± 0.2[a]
Alkaline phosphatase, U/g	108.0 ± 4.6	124.0 ± 5.7[a*]	158.0 ± 4.7[ab]	152.0 ± 5.4[ab]	171.0 ± 4.2[a]	159.0 ± 3.8[ab*]	136.0 ± 5.9[ab]
Acid phosphatase, U/g	102.0 ± 6.9	177.0 ± 9.2[a]	151.0 ± 6.6[ab*]	162.0 ± 7.7[a]	154.0 ± 3.8[a]	120.0 ± 7.2[b]	151.0 ± 7.4[a]

[a] significant over control at 1%, [b] significant over corresponding defatted meal at 1%, * indicate significance at 5%. All values are mean ± SEM on fresh tissue basis. Enzymes units (U) are as defined in the references given in the text

could provide a biochemical index of the health of the organ of that tissue. In view of this, tissues from pancreas, liver and small intestine were assayed for certain enzymes (Table 2). Feeding defatted soybean meal to rats significantly raised the activities of protease, trypsin and reduced the activity of amylase of the pancreas and small intestine as compared to the control group. Furukawa et al (1987) also noted an increase of intracellular zymogen granules in pancreas, which may be the cause of increase in trypsin and protease activities reported in the present study. There were also significant increases in the activities of alkaline and acid phosphatases of liver and small intestine. Increased activities of acid phosphatase of liver, trypsin and acid and alkaline phosphatases of small intestine and reduced activity of amylase of pancreas and small intestine of defatted mothbean group were also observed.

Since trypsin inhibitors were present in the defatted black-soybean and mothbean meals, the observed variation in the enzymic activity of pancreas, liver and small intestine could be due to them. In order to test this, defatted processed meals, which were free from trypsin inhibitors, were fed to rats and the results are presented in Table 2. While comparing the activities of amylase, protease and trypsin of the pancreas and small intestine amongst the various experimental groups, it was found that the differences between the pancreas of the control group and the processed meal groups were non-significant. The differences between the small intestine of the control group and the buffer-extracted meal group were also non-significant. The differences between the small intestine of the acetylated soybean meal group and control were slight. The amylase and trypsin activities of the small intestine of the processed mothbean meal group were not different from those of the defatted meal group. The activities of acid and alkaline phosphatases, however, behaved differently from those of other enzymes. Excepting the acid phosphatase activity of liver of the acetylated soybean meal group and of small intestine of buffer-extracted mothbean meal group, activities of both the phosphatases in all other groups were

higher than the control group. A perusal of the data reveals that the magnitude of changes observed in the mothbean meal group was much lower than the black-soybean meal group, which can be explained on the basis of the presence of a very high amount of trypsin inhibitor (17 times) in defatted soybean meal compared to mothbean meal.

The differential results obtained by feeding raw defatted and processed meals devoid of trypsin inhibitor to rats, were indicative of the involvement of trypsin inhibitor in the changes produced in pancreas, liver and small intestine. The acid and alkaline phosphatase activities, however, did not appear to be appreciably affected by the inhibitor. Milder processing methods used in this study avoid denaturation of the protein and reaction between the chemical components of the meal. Thus, the restoration of partial or total activities observed in the present study could only be due to the removal of trypsin inhibitor and not due to loss of any other nutrient through chemical reaction (Lee et al. 1979). Acetylation of meals was used not only to see the efficiency of chemical processing, but also to control the loss of protein.

References

AOAC (1980) Official Methods of Analysis, 13th edn. Association of Official Agricultural Chemists, Washington, DC

Bernfeld P (1955) Amylases, α and ß. In: Colowick SP, Kaplan NO (eds) Methods in Enzymology. Vol I, Academic Press, New York, pp 149–158

Carr JJ (1953) Alkaline and Acid phosphatase. In: Reiner M (ed) Standard Methods of Clinical Chemistry, Vol 1, Academic Press Inc, New York, pp 75–83

Chohan AK, Hamilton RMG, McNiven MA, Macleod JA (1993) High protein and low trypsin inhibitor varieties of full fat soybeans in broiler chicken starter diets. Can J Anim Sci 73: 401–409

Furukawa F, Toyoda K, Abe H, Hasegawa R, Sato H, Takahashi M, Hayashi Y (1987) Short-term effects of feeding crude soybean trypsin inhibitor on pancreas of rat, hamster and mouse. Bull Natl Inst Hyg Sci (Tokyo), pp 46–50

Ge YC, Morgan RGH (1993) The effect of trypsin inhibitor on the pancreas and small intestine of mice. Brit J Nutr 70: 333–345

Kakade ML, Hoffa DE, Liener IE (1973) Contribution of trypsin inhibitors to the deleterious effect of unheated soybean fed rats. J Nutr 103: 1772–1778

Lee S, Sen LC, Clifford AJ, Whitaker JR, Feeney RE (1979) Preparation and nutritional properties of casein covalently modified with sugars. Reductive alkylation of lysine with glucose, fructose or lactose. J Agric Food Chem 27: 1094–1098

Lee H, Ham IK, Choi YJ, Yun CH (1991) Effect of trypsin inhibitor content in soybean meal on turkey performance and amino acid digestibility. Korean J Anim Sci 33: 762–767

Liener IE, Kakade ML (1980) Protease inhibitors. In: Liener IE (ed) Toxic Constituents of Plant Foodstuffs. 2nd edn. Academic Press Inc New York, London. pp 7–72

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with Folin-Phenol reagent. J Biol Chem 193: 265–275

Marquardt RR, Campbell LD, Ward T (1976) Studies with chicks on the growth depressing factor(s) in faba beans *(Vicia faba L* var *'Minor')* J Nutr 106: 275–284

Mehta SL, Simlot MM (1982) Purification and characterization of trypsin inhibitor from mothbean *Phaseolus aconitifolius.* Indian J Biochem Biophys 19: 403–407

Melmed RN, El-Aaser AAA, Holt SJ (1976) Hypertrophy and hyperplasia of the neonatal rat exocrine pancreas induced by orally administered soybean trypsin inhibitor. Biochim Biophys Acta 421: 280–288

Nitsan Z, Liener IE (1976) Enzymic activities in the pancreas, digestive tract and faeces of rats fed raw or heated soy flour. J Nutr 106: 300–305

Nitsan Z, Bruckental I (1977) Effect of treating soybean meal with formaldehyde on the activity of enzymic inhibitors and protein utilization in the chick. J Anim Sci 44: 998–1003

Rackis JJ, McGhee JE, Booth AN (1975) Biological threshhold levels of soybean trypsin inhibitors by rat bioassay. Cereal Chem 52: 85–92

Ramamani S, Chandrasekhara HN, Narsimhamurthy K (1996) Efficiency of inactivation of trypsin inhibitors and haemagglutinins by roasting of soybean *(Glycin max).* J Food Sci Technol 33: 197–201

Simlot MM, Feeney RE (1966) Relative reactivities of chemically modified turkey ovomucoids. Arch Biochem Biophys 113: 64–71

Subbulakshmi G, Ganesh Kumar K. Venkataraman LV (1976) Effect of germination on carbohydrates, proteins, trypsin inhibitor and haemagglutinins in horsegram and mothbean. Nutr Rep Int 13: 19–31

Zhang Y, Parsons CM, Weingartner KE, Wijeratne WB (1993) Effects of extrusion and expelling on the nutritional quality of conventional and Kunitz trypsin inhibitor-free soybeans. Poultry Sci 72: 2299–2308

Received 16 March 1995; revised 4 February 1997; accepted 6 February 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 3, 212-217

Effect of Homogenization on Sensory Quality and Rheological Characteristics of Pulp and Beverages from Ripe 'Dushehari' Mangoes

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Homogenization of pulp, squash, nectar and ready-to-serve beverage from ripe 'Dushehari' mangoes reduced pulp particle size, which improved the consistency and acceptability of the beverages. The beverages were stored at 4 ± 1°C, 28 ± 2°C and 38 ± 2°C for 30 days. Storage at 4 ± 1°C was found to ensure maximum retention of chemical and sensory characteristics. All mango products were non-Newtonian pseudoplastic fluids. An integrated model $\ln K = b_0 + b_1 \ln P + b_2 \ln S + b_3/T$, was found to predict satisfactorily the combined effects of temperature (T, Kelvin), pulp content (P, %) and total soluble solids (S, %) on 'K' for unhomogenized or homogenized mango products.

Keywords: Beverages, Homogenization, Mango, Rheological characteristics, Sensory quality, Storage.

Squash, nectar and ready-to-serve (RTS) beverages are important products prepared from mango pulp (Jagtiani et al. 1988). Several quality characteristics of these products, viz., consistency/ viscosity, mouthfeel, homogeneity, pulp separation and appearance are affected by the size of pulp particles. Size of pulp particles is controlled by the mesh size of sieves used in pulper/finisher (Jagtiani et al. 1988). Viscosity characteristics of mango pulp and beverages were studied be several workers (Gunjal and Waghmere 1987; Ranganna 1986; Rao et al. 1974, 1985; Siddalingu et al. 1985). They found that (i) the products were non-Newtonian pseudoplastic fluids and (ii) their rheological constants (K, consistency coefficient and n, flow behaviour index) varied with variety of mango, type of the product and total soluble solid contents (TSS). Temperature dependence of 'K' was described with a simple Arrhenius type equation. These studies were conducted with unhomogenized mango products such as, pulp, juice and nectar. But, homogenization is known to alter the product characteristics (Prentice 1984). In the case of apricot *puree*, homogenization has been reported to alter product thixotropy and increase the value of 'K' (Duran and Costall 1985), whereas it caused a significant reduction in the viscosity of orange juice and concentrate (Crandall and Davis 1991; Vitali and Rao 1984). This paper reports the effect of homogenization on the viscosity, acceptability and storage characteristics of mango pulp and its beverages (squash, nectar and RTS beverage).

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Materials and Methods

Soft ripe mangoes (21° Brix TSS) of 'Dushehari' variety were washed in water, peeled manually and pulped in a pulper fitted with 1.5 mm screen. The pH of the pulp (21° Brix TSS) was adjusted to 4.1 by adding citric acid (0.5% w/w of pulp) and the acidified pulp was heated for 2 min at 93°C in a steam-jacketted kettle to inactivate pectinases and other enzymes and air-cooled (Nath and Ranganna 1980). It was preserved with 1730 ppm potassium metabisulphite (1000 ppm SO_2), and stored in 20 litre plastic containers under ambient conditions (26 ± 3°C), till required for use.

Homogenization : Mango pulp was homogenized at pressures of 2000-4000 psig in a homogenizer (Gaulin Corpn., Massachusetts, USA, Type 120M3, 5TBS, SMD). Higher pressures could not be tried because it caused choking. To assess the efficiency of homogenization, size of pulp particles was measured by the microscopic method (Terence 1968). Pulp particles of larger size tend to settle down; a phenomenon which is clearly visible in mango beverages such as RTS beverage. This tendency of pulp separation was measured as volume of the centrifugate obtained by centrifuging 25ml pulp sample at 6000 rpm for 6 min.

Beverages : Beverages conforming to the FPO specifications (Anon 1975) were prepared, using mango pulp homogenized at 4000 psig. Sugar and citric acid were dissolved in requisite amount of water, syrup was filtered through muslin cloth, cooled and mixed well with the pulp (Table 1). Beverages prepared similarly from unhomogenized

TABLE 1. INGREDIENTS* USED FOR PREPARING MANGO BEVERAGES

Beverage	Mango pulp, %	Cane sugar, %	Citric acid, %
RTS beverage	10.0	12.0	0.53
Nectar	20.0	16.0	0.48
Squash	25.0	40.5	0.58

* Remaining part was water

pulp were divided into two lots – one lot was homogenized at 4000 psig and the other unhomogenized portion was used as control. Beverages were brought to boil, filled hot into 150 ml glass bottles, sealed and kept for subsequent studies.

Storage studies : As pulps homogenized at 4000 psig were found to give the most acceptable beverages, storage studies were carried out with them only. Bottled beverages were stored at 4±1°C, 26±2°C, and 38±2°C for 30 days. Storage changes in TSS, pH, acidity, total and reducing sugars and ascorbic acid were followed. Sugars were estimated by Lane and Eynon method and ascorbic acid by the indophenol dye titration method (Ranganna 1986).

Sensory evaluation : Composite scoring method (Ranganna 1986) was used for carrying out sensory evaluation. At first, the effect of homogenization pressure on the sensory attributes of mango beverages was assessed. For this purpose, fresh samples were scored for flavour, colour, consistency and absence of defects. The scores for the four attributes given by a panelist were compounded in the ratio of 15:30:40:15 to arrive at a composite score. Consistency was given the maximum weightage, because it is known to be influenced most by homogenization.

However, storage studies were carried out with the samples prepared from homogenized pulp, in which not much difference was expected in consistency. Therefore, less weightage was given to it. The maximum scores assigned to taste, aroma, colour and consistency were 40, 30, 15 and 15, respectively for arriving at the composite scores. Sensory data were subjected to the analysis of variance to determine significance of difference at $P < 0.05$.

Viscosity : A Brookfield Synchroelectric Viscometer (LVT model) was used to measure rheological constants of mango pulp and beverages (Charm 1963). Spindle No. 1 was used for RTS beverage and nectar and spindle No. 4 was used for pulp and squash. Power law model (Eq. 1) is a general expression correlating shear stress (τ) and shear rate (γ).

$$\tau = K\gamma^n \quad \ldots\ldots (1)$$

Where 'K' is consistency coefficient and 'n' is flow behaviour index.

To study the effect of temperature on the rheological constants (K and n), measurements were made over a temperature range of 23–85°C in a water bath and calculations were made using Eq. (1) after neglecting the yield stress. The data were fitted to Eq. (2), which is an Arrhenius model (Gunjal and Waghware 1987).

$$\ln K = \ln K_a + E_a/RT \quad \ldots\ldots (2)$$

Eq (2) does not involve 'n'. Therefore, Christiansen-Craig model (Eq. 3), having both rheological constants (K and n) was used in this study (Rao 1986). Since 'n' is not very sensitive to temperature variations and 'K' is strongly dependent upon temperature (Bhamidipati and Singh 1990). it was replaced with $\bar{n}$ (average of n for a sample at different temperatures), as was done by Harper and El Sahrigi (1969) and the data were fitted in the (Eq. 4) so obtained.

$$\ln K = \ln K_{ac} + (E_{ac}/RT)^n \quad \ldots\ldots (3)$$

$$\ln K = \ln K_{am} + (E_{am}/RT)^{\bar{n}} \quad \ldots\ldots (4)$$

Where K_a is a constant, E_a is activation energy, R is gas constant and T is absolute temperature of the sample in Kelvin. Subscript 'c' and 'm' to K_a and E_a stand for the constants in Eq. (3) and Eq. (4), respectively.

Rheological characteristics of fluid foods are influenced by their pulp content (P, %), soluble solids (S, %) and temperature (T, Kelvin). Bhamidipati and Singh (1990) developed Eq. (5) to correlate consistency coefficient with P, S and T.

$$\ln K = b_0 + b_1 \ln P + b_2 \ln S + b_3/T \quad \ldots (5)$$

Where b_0, b_1, and b_3 are constants.

Regression analysis of equation (1) to (5) was done on computer (model EC–486, EC IL, Hyderabad) to calculate various constants and to test their fit.

Results and Discussion

Ripe 'Dushehari' mangoes contained on an average 70% pulp of TSS 21°Brix. Peels and stones constituted 30% of fruits. Pulp was adjusted to a pH of 4.1 and 0.53% acidity. Sugars constituted 91.4% of the mango solids, of which 78.1% were in the non-reducing form. Pulp contained 32.5 mg% AA.

TABLE 2. EFFECT OF HOMOGENIZATION PRESSURE ON PARTICLES SIZE AND SEPARATION* IN MANGO PULP AND SENSORY CHARACTERISTICS OF MANGO BEVERAGES

Homogenization pressure for pulp, psig	Mango pulp		Mean sensory score					
	Particle size, mm	Separation*, %	RTS beverage		Nectar		Squash	
			CS(40)**	OA(100)***	CS (40)**	OA(100)**	CS (40)**	OA(100)**
0	1.17 ± 0.17	55.0 ± 0.50	27[a]	72[a]	32[a]	79[a]	28[a]	75[a]
2000	0.70 ± 0.05	37.3 ± 0.30	28[a]	74[a]	30[b]	75[b]	31[b]	79[b]
3000	0.44 ± 0.02	24.7 ± 0.32	30[b]	77[b]	33[c]	79[a]	32[b]	81[c]
4000	0.42 ± 0.01	20.5 ± 0.25	34[c]	82[c]	35[d]	86[c]	34[c]	84[d]
CD at P <0.05	-	-	1.34	2.79	1.49	2.87	1.76	2.87

* Centrifuged for 6 min. at 6000 rpm; ** maximum score; CS: Consistency score; OA : Overall acceptability, Sensory scores followed by same alphabet do not differ significantly at p < 0.05

Effect of homogenization : The average size of unhomogenized pulp particles was 1.17±0.37mm (Table 2). Homogenization reduced the pulp particle size significantly, but the difference between size of pulp particles, homogenized at 3000 and 4000 psig were non-significant (CD at P < 0.05:0.12). Decrease in the particle size was accompanied by a reduction in the tendency of pulp separation as expected from Stoke's law. Consequently, pulp separation was only 20.50 ± 0.25% for pure homogenized pulp at 4000 psig, as compared to 55.0 ± 0.50% separation observed for the unhomogenized pulp (Table 2).

Overall acceptability scores of the mango beverages prepared from unhomogenized pulp were 72–79, out of a maximum score of 100. It was found to increase significantly to 82–86, when the samples were prepared from pulp homogenized at 4000 psig (Table 2). Since homogenization did not produce any significant change in the sensory scores for flavour, colour or absence of defects, scores for only consistency (CS) and overall acceptability (OAS) are included in Table 2. The pattern of changes in CS and OAS was similar and for both the parameters, scores were found to increase with an increase in the homogenization pressure.

Storage studies : Storage for 30 days reduced the overall acceptability scores of the beverages prepared from mango pulp, homogenized at 4000 psig (Table 3). Changes were significant in all the samples, except for the RTS beverage stored at 4 ± 1°C. Non-significant changes in the latter may be due to its low pulp content (10%). Reduction in the OAS was greater in the samples stored at higher temperatures.

Chemical constituents of the beverages changed during storage at all the three temperatures, changes being maximum at 38±2°C (Table 3). Storage at 38°C lowered the pH and increased the acidity significantly. Similar results were reported earlier

TABLE 3. EFFECT OF 30 DAYS STORAGE AT 38 ± 2°C ON CHEMICAL CONSTITUENTS AND OVERALL ACCEPTABILITY OF BEVERAGES FROM MANGO PULP HOMOGENIZED AT 4000 PSIG

Chemical constituents	Pulp	RTS beverage		Nectar		Squash	
		Fresh	Stored	Fresh	Stored	Fresh	Stored
TSS, °Brix	21.00	14.00	14.00	20.00	20.00	45.00	45.00
pH	4.10	4.00	3.82	3.99	3.92	3.88	3.71
Acidity, % as citric acid	0.53	0.52	0.62	0.48	0.53	0.56	0.67
Sugars, %							
Total	19.21	12.89	12.59	18.53	18.85	42.00	39.33
Reducing	3.40	4.24	6.74	6.36	10.13	13.74	18.25
Ascorbic acid, mg%	32.50	32.50	17.90	36.90	21.30	39.30	21.00
Total SO_2, ppm	-	89.30	62.00	118.00	61.30	341.00	243.00
OA scores for samples stored at control[1]	-	-	84[a]	-	85[a]	-	85[a]
4 ± 1°C	-	-	82[a]	-	77[b]	-	81[b]
28 ± 2°C	-	-	73[b]	-	74[c]	-	76[c]
38 ± 2°C	-	-	65[c]	-	69[d]	-	71[d]
CD at P<0.05	-	-	2.70	-	2.44	-	2.14

OA : Overall acceptability score out of a maximum score of 100; [1] Beverage prepared freshly from frozen homogenized pulp; Scores followed by same alphabet do differ significantly at P < 0.05

TABLE 4. REGRESSION COEFFICIENTS FOR DIFFERENT MODELS (EQ. 2 & 4) SHOWING RELATIONSHIP BETWEEN CONSISTENCY COEFFICIENT (K), FLOW BEHAVIOUR INDEX (n or $\bar{n}$) AND TEMPERATURE (T) IN THE TEMPERATURE RANGE OF 290 TO 358 KELVIN

Product	n	$\bar{n}$	Equation, ln K=	df	R^2	Error MS	Model
Pulp							
UHP	0.285–0.299	0.293	0.528+904.7/T	3	0.949	0.0020	Eq. 2
			$-5.926+50.35/T^{0.293}$	3	0.940	0.0023	Eq. 4
HP	0.178–0.250	0.209	1.262+958.2/T	3	0.992	0.0003	Eq. 2
			$-9.655+46.39/T^{0.209}$	3	0.993	0.0003	Eq. 4
Squash							
UHPB	0.164–0.402	0.312	-5.463+2103.3/T	4	0.986	0.0044	Eq. 2
			$-19.731+126.39/T^{0.312}$	4	0.981	0.0059	Eq. 4
HPB	0.365–0.422	0.391	-2.962+1291.6/T	4	0.954	0.0036	Eq. 2
			$-9.000+95.84/T^{0.391}$	4	0.947	0.0041	Eq. 4
HB	0.338–0.416	0.376	-3.338+1477.1/T	4	0.994	0.0006	Eq. 2
			$-10.775+105.8/T^{0.376}$	4	0.993	0.0007	Eq. 4
Nectar							
UHPB	0.311–0.558	0.431	-8.789+2672.6/T	4	0.978	0.0112	Eq. 2
			$-19.610+230.42/T^{0.431}$	4	0.974	0.0132	Eq. 4
HPB	0.311–0.594	0.472	-6.269+1981.2/T	4	0.994	0.0017	Eq. 2
			$-13.516+205.12/T^{0.472}$	4	0.988	0.0035	Eq. 4
HB	0.400–0.533	0.460	-6.493+2068.5/T	3	0.999	0.0001	Eq. 2
			$-14.005+198.0/T^{0.460}$	3	0.999	0.0002	Eq. 4
RTS beverage							
HB	0.443–0.578	0.508	-9.513+2437.0/T	3	0.991	0.0033	Eq. 2
			$-18.374+309.3/T^{0.508}$	3	0.995	0.0019	Eq. 4

UHP : Unhomogenized pulp; HP: Homogenized pulp, UHPB: Beverage from unhomogenized pulp; HPB: Beverage from homogenized pulp; HB: Homogenized beverage.

Eq. 2: Arrhenius model; Eq. 4: Modified Christiansen-Craig model

by Palaniswamy et al (1974) for mango pulp and squash. During storage, total sugar contents of the beverages remained almost unchanged, but the amount of reducing sugars increased significantly due to acid hydrolysis of sucrose. Fresh beverages, which were fortified with ascorbic acid (AA) lost 42.4 to 47.9% AA during this storage period as compared to a loss of upto 40% reported earlier by Sahni and Khurdia (1989) for 'Dushehari' mango nectar. Total SO_2 level also decreased by 28.4 – 48.1% during this period.

Rheological characteristics : Apparent viscosity of unhomogenized and homogenized samples decreased with an increase in the spindle speed, i.e., shear rate, indicating that mango pulp and its beverages are non-Newtonian pseudoplastic fluids (Rao 1986; Gunjal and Waghmare 1987) and homogenization did not change this characteristic.

The rheological constants for RTS beverage could be calculated only for the homogenized products. For the other types of RTS beverages, the viscometer dial readings at different spindle depths, i.e., at lower torques and spindle speeds (varying shear rates) needed for computation were too small to be recorded accurately (Table 4). The values of 'n' for the mango products are in the range of 0 to 1 as expected for pseudoplastic fluids (Table 4). Values of 'n' of a product at different temperatures were averaged to obtain average 'n' or ' $\bar{n}$'. Values of $\bar{n}$ was highest (0.508) for homogenized RTS beverage and decreased to 0.209 for mango pulp. This decreasing trend indicates that pseudoplasticity

TABLE 5. REGRESSION EQUATION BASED ON EQ. (5) SHOWING RELATIONSHIP BETWEEN CONSISTENCY COEFFICIENT (K), PULP CONTENT (P, %), TSS (S, %) AND TEMPERATURE (T, KELVIN) OF MANGO PRODUCTS

Product	Equation (ln K =)
Unhomogenized pulp/squash/nectar	$-18.260 + 3.535^{**} \ln P + 3.299^{**} \ln S + 0.003^{**}/T$
	(R^2 : 0.993 Error MS : 0.025)
Homogenized pulp/squash/nectar/RTS beverage	$-15.993 + 3.281^{**} \ln P + 3.122^{**} \ln S + 0.003^{**}/T$
	(R^2 : 0.997 Error MS : 0.023)
Squash and nectar from homogenized pulp	$-2.134 + 3.095 \ln P + 3.356 \ln S + 0.003^{**}/T$
	(R^2 : 0.988 Error MS : 0.009)

of mango products increased with an increase in pulp content and TSS. The phenomenon of increase in the viscosity upon reduction in particle size as observed during homogenization, has been explained by Bhamidipati and Singh (1990), using hydrodynamic approach. Consistency coefficient without yield stress (K dyn $cm^{-2}S^n$) was 60.5 for unhomogenized pulp and 1.216 for unhomogenized nectar (Table 5), as compared to the reported values of 23.56 - 299.9 and 1.64 - 2.09, respectively (Gunjal and Waghmare 1987; Ranganna 1986; Rao et al. 1974, 1985). Observed differences may be due to differences in mango varieties used to prepare the product or TSS or the product.

Values of K obtained from Eq. (1) were very sensitive to the variations in the viscosity of mango products. Therefore, it was used to assess the effect of temperature on rheological constants (Table 4). Christiansen-Craig model (Eq. 3) gave a poor fit of the data (R^2 0.164 - 0.874). But, Arrhenius model (Eq. 2) and the modified Christiansen-Craig model (Eq. 4) showed highly significant positive correlation (R^2 0.949 - 0.999 and 0.940 - 0.999) and low error mean squares, indicating that both models described the dependence of viscosity on temperature equally well. Fig. 1A is an Arrhenius plot based on Eq. (2) and Fig. 1B is a modified

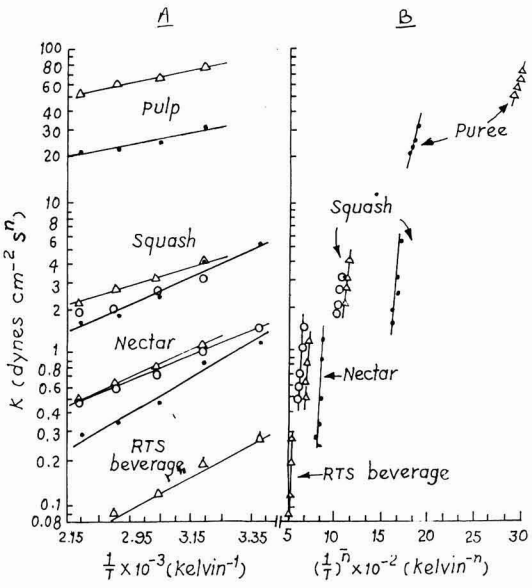

Fig 1(A). Arrhenius plot, and (B) modified Christiansen-Craig plot showing effect of temperature (T) on consistency coefficient (K) of 'Dushehari' mango pulp and beverages, when n is flow behaviour index at a temperature and $\bar{n}$ is its average value (unhomogenized product -●-; homogenized product -Δ-; beverage from homogenized pulp -0-).

Christiansen-Craig plot based on Eq. (4). In general, the slope of these curves increased as the pulp contents of the product decreased, i.e., viscosity of nectar was more temperature dependent than that of squash or pulp. According to Prentice (1984), viscosity of sugar solution is more dependent on temperature than fruit juices and *purees* due to the presence of particulates in the latter.

In order to develop an integrated equation for describing the combined effect of pulp content, TSS and temperature on viscosity, the mango products were divided into three groups of homogeneous products - unhomogenized products (pulp and beverages), homogenized products (pulp and beverages), and beverages prepared from homogenized pulp (Table 5). Regression of the corresponding sets of data into Eq. (5) gave high values of coefficients of multiple regression (R^2 0.988 - 0.977) and low error mean squares (0.0090 - 0.0248). Further, T-values for the three constants of Eq. (5) were highly significant, except for the products from the homogenized pulp. It shows that consistency coefficient, K is strongly dependent upon pulp content, TSS and temperature of the mango products. Therefore, Eq. (5) $\ln K = b_0 + b_1 \ln P + b_2 \ln S + b_3/T$, can be used to predict satisfactorily the effect of temperature on the viscosity of a group of homogeneous mango products, irrespective of the differences in their pulp content or TSS.

Conclusions

Investigations carried out to determine (i) the effect of homogenization and viscosity and (ii) effect of temperature, pulp content and total soluble solids on rheological characteristics of mango beverages have shown that homogenization increased the consistency and improved the acceptability of the beverages.

Consistency coefficient was found to depend upon temperature, pulp content and total soluble solids of the beverage.

References

Anon (1975) The Fruit products Order 1955 (As amended upto 31st December 1974). Indian Food Packer 29 (1): 46-82

Bhamidipati S, Singh RK (1990) Flow behaviour of tomato sauce with or without particulates in tube flow. J Food Process Eng 12: 275-293

Charm SE (1963) Effect of yield stress on the power law constants of fluid food materials, determined in low shear rate viscometers. Ind Eng Chem Process Design Dev 2: 62-65

Crandall PG, Davis KC (1991) Viscosity reduction and reformation of structure in orange concentrate as affected by homogenization within commercial TASTE evaporators. J Food Sci 56: 1360–1364

Christiansen EB, Craig Jr SE (1962) Heat transfer to pseudoplastic fluids in laminar flow. AICE J 8: 154–160

Duran L, Costall E (1985) Influence of homogenization on the rheological behaviour of apricot *puree*. Acta Alimenteria 14: 201–210

Gunjal BB Waghmare NJ (1987) Flow characteristics of pulp, juice and nectar of 'Baneshan' and 'Neelum' mangoes. J Food Sci Technol 24: 20–23

Harper JC, El Sahrigi AF (1965) Viscometric behaviour of tomato concentrates. J Food Sci 30: 470–476

Jagtiani J, Chan Jr HT, Sakai WS (1988) Tropical Fruit Processing. Academic Press, Inc. New York, pp 45–97

Nath Nirankar, Ranganna S (1980) Determination of thermal process schedule for 'Totapuri' mango. J Food Technol 15: 251–264

Palaniswamy KP, Muthukrishnan CR, Shanmugavelu KG (1974) Physio-chemical characteristics of some varieties of mango. Indian Food Packer 28 (5): 12–19

Prentice JH (1984) Measurements in the Rheology of Foodstuffs, Elsevier Applied Science Publishers, London and New York

Ranganna S (1986) Handbook of Analysis and Quality Control for Fruit and Vegetable Products, 2nd edn, Tata McGraw-Hill Publishing Company Ltd. New Delhi pp 9, 12, 105, 630

Rao KI, Eipeson WE, Rao PNS, Patwardhan MV, Ramanathan PK (1985) Rheological properties of mango pulp and concentrates. J Food Sci Technol 22: 30–33

Rao MA (1986) Rheological properties of fluid foods. In: Rao MA, Rizvi SSH (eds). Engineering Properties of Foods. Marcel Dekker Ins., New York, pp 1–47

Rao MA, Otoya Palomino LN, Bernhardt LW (1974) Flow properties of tropical fruits *purees*. J Food Sci 39: 160–161

Sahni CK, Khurdiya DS (1989) Effect of ripening and storage temperature on the quality of mango nectar. Indian Food Packer 43 (6): 5–11

Siddalingu, Srinivasan B, Padival RA, Ranganna S (1985) Determination of thermal process schedule for canned mango, papaya and guava pulps. Acta Alimentaria 14: 331–342

Terence A (1968) Particle Size Measurement, Chapman Hall Ltd., London, pp 45–59

Vitali AA, Rao MA (1984) Flow properties of low-pulp concentrated orange juice : Serum viscosity and effect of pulp content. J Food Sci 49: 876–881

Received 24 April 1995; revised 18 March 1997; accepted 21 March 1997

Determination of Degree of Cooking of Vegetables By Compression Testing

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Degrees of cooking green peas, carrot, radish and knol-khol were determined in terms of relative hardness. The mean peak compression force values at various cooking time intervals were determined using an INSTRON-Universal Testing Machine (Model 4301). The peak compression force was correlated with the cooking time by developing curvilinear regression analysis. To reduce the effect of absolute values, the peak compression force was converted into dimensionless numbers as relative hardness and was correlated with the cooking times. Finally, empirical equations for degree of cooking was evolved in terms of relative hardness. The coefficients of fit were determined, using R^2 and mean modulus (P) values were found in the range of 0.988 to 1 and 5 to 8, respectively.

Keywords: Texture studies, Vegetable, Instron, Degree of cooking, Relative hardness.

Vegetables form an important and essential component of human diet, as they constitute important sources of vitamins, minerals and dietary fibres. Most of the vegetables can be consumed raw. But not all vegetables can be consumed raw. They retain a majority of their nutrients without being processed. Also, before any of the preservation techniques like canning, freezing and dehydration or in the preparation of cooked dish, vegetables have to be either blanched or cooked. During the process of cooking, some chemical and structural changes take place in vegetables. One of them is thermal softening. Excessive softening caused during cooking renders some foods unpalatable (Huang and Bourne 1983). Kostaropoulos (1981) studied the freshness of vegetables and derived an empirical expression for textural studies of vegetables. Schutz et al (1974) have reported that consumers rate flavour and texture of vegetables as the most important sensory attributes.

In quality control and in research and development, there is requirement for rapid and easy instrumental measurement of quality attribute to bypass, wherever possible the more expensive and time consuming sensory panel analysis (Sawyer 1971). However, the instrumental method should highly correlate with the sensory analysis of the quality attribute. Usually, calibration is carried out when an expensive, time consuming and precise measure of some physical characteristics is replaced by subjective measure, one that is more convenient or economical. This phenomenon occurs very often in food technology applications.

Commonly, sensory panel members evaluate firmness and degree of cooking by squeezing the vegetables between their fingers (Van Loey et al. 1994). The nearest instrumental method, for the above objective measurement of degree of cooking is the uniaxial compression testing. Parallel plate uniaxial compression using INSTRON-UTM is commonly used for food texture investigations. Huan and Bourne (1983) have determined the rate of softening in several canned vegetables during the retort process. But, they have not correlated their results with degree of cooking. Bera et al (1990) have applied INSTRON tester (Model 4301) to study the effect of soaking on degree of cooking of faba bean *dhal*.

But with respect to vegetables, no attempt has been made by previous researchers to correlate the textural properties with the degree of cooking. Mittal (1994) has studied the thermal softening of potatoes and carrots and observed that more data on thermal softening of many foods are needed. The objective of the present study was, therefore, to develop empirical formulae to estimate degree of cooking of vegetables in terms of mean peak compressive force.

Fresh 'Arkel' variety green peas (*Pisum sativum*) were shelled manually, graded to a uniform size of 9.5±1 mm and three other vegetables; carrot (*Daucus carota*) 'Desi' variety, knol kol (*Brassica oleracea*) 'Caolorapa' variety and 'White and stout' variety radish (*Raphamus sativus*) were obtained commercially on the day of the experiment and washed. Carrot, knolkol and radish were diced into pieces of 10 mm, 12.5 mm, 30 mm in height and

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25 mm in diameter, using a cork borer and a sharp knife.

Cooking method : The vegetables were cooked in water at 98±2°C. This temperature was selected because the softening rate is lowere than that at high temperatures used in vegetable canning and this allows more data points to be obtained in the curvilinear portion of the softening curve. Bourne (1987) has concluded that both high and low temperature treatments have quantitatively similar softening curves. Washed vegetables (100±25 g) were heated in 500 ml glass beaker. The contents were continuously agitated, using a laboratory strirrer to reduce the temperature gradient during heating to minimize the difference in heat treatment at the surface and at the centre of cooking container. Samples were cooked for pre-determined heating times. The process times included the range of distinct over-cooking. At the end of the cooking period, the cooked vegetables were cooled in running water at room temperature. The surface water was removed by pressing gently between two hand-made filter papers. Samples of cooked vegetables were taken in replicates of four from the batch to determine the statistical variations of the mean compression force.

Measurement of peak force : The uniaxial compression tests were performed on an INSTRON (Model 4301) universal testing machine with a load cell of 1000 N to record the force exerted on the sample. The cylindrical sample of cooked vegetable was compressed to determine the mean peak compressive force. The INSTRON UTM consisted of a stationary bottom plate and upper movable crosshead. The movable crosshead was connected to a load cell to record the force exerted on the sample. All testing was done with the INSTRON installed inside an environmental room maintained at 25°C. The cooked samples were taken in stainless steel cylinder of dimensions 50 mm diameter and height. A plunger was attached to the upper movable crosshead. The plunger moved in close tolerance within the sample cylinder. The sample cylinder was placed on the stationary plate and cooked vegetable was compressed to determine the peak compression force.

Kostaropoulos (1981) has established that the compression test fits better to the related sensory analysis. Also, these results are directly applicable to on-line control systems and simulating compression is more appropriate. Hence, compression testing was used. Following Boyd and Sherman (1975), a crosshead speed of 50 mm/min and a compression to 25% of the original height was selected except for green peas for which 50% compression was selected. This was, because at 25% compression for green peas, the data of six replicates did not match properly and the deviation was large (data not reported). The peak compression force was determined as the mean value of 4 replicates from the same batch. Experiments were repeated at different time intervals of 2, 4 and 6 min. Table 1 gives data for 4 vegetables at different time intervals. The mean values with standard deviations are reported.

Computation: Calculations were carried out on an IBM compatible micro-computer. The Grapher software and Quatro Pro software were used for regression analysis. The regression equations were estimated, using three different least square methods, viz., exponential, non-linear regression, multiple regression of polynomial. However, large confidence intervals made the result of first two methods not satisfactory and it was found that the

TABLE 1. MEAN PEAK COMPRESSION FORCE FOR VEGETABLES

Cooking time (t), min	Green peas			Carrot			Knol-khol			Radish		
	Mean peak force, N	Relative hardness, N/N	Degree of cooking, %	Mean peak force, N	Relative hardness, N/N	Degree of cooking, %	Mean peak force, N	Relative hardness, N/N	Degree of cooking, %	Mean peak force N	Relative hardness, N/N	Degree of cooking, %
0	860 ± 35	1.00	00.0	963 ± 82	1.00	00.0	713 ± 32	1.00	00.0	334 ± 61	1.00	00.0
5	425 ± 20	0.49	50.0	400 ± 23	0.41	71.0	340 ± 57	0.48	61.8	274 ± 28	0.82	20.9
10	376 ± 09	0.44	72.0	325 ± 31	0.34	76.1	233 ± 44	0.33	79.6	190 ± 08	0.59	50.2
15	294 ± 12	0.35	84.0	225 ± 39	0.23	88.0	175 ± 48	0.25	89.2	149 ± 08	0.45	64.4
20	232 ± 42	0.27	94.0	199 ± 07	0.21	91.1	130 ± 44	0.18	97.0	118 ± 11	0.28	75.4
25	219 ± 17	0.25	96.0	142 ± 13	0.15	98.0	110 ± 11	0.15	100.0	94 ± 14	0.28	83.5
30	192 ± 13	0.23	98.0	125 ± 14	0.13	100.0	-	-	-	68 ± 04	0.20	92.6
35	190 ± 09	0.22	100.0	-	-	-	-	-	-	61 ± 05	0.15	99.2
40	-	-	-	-	-	-	-	-	-	47 ± 23	0.14	100.0

The data are average of 4 replications. The values after ± sign are standard deviations

polynomial equations predicted more accurately. Hence, the results of other two equations are not reported.

The polynomial equation of 4h order was obtained in the form shown below

Mean peak force (Y) v/s cooking time (X)

$$Y = a_0X^0 + a_1X^1 + a_2X^2 + a_3X^3 \quad \text{..... (1)}$$

Relative hardness (Y) v/s cooking time (X)

$$Y = b_0X^0 + b_1X^1 + b_2X^2 + b_3X^3 \quad \text{..... (2)}$$

Degree of cooking (Y) v/s relative hardness (X)

$$Y = c_0X^0 + c_1X^1 + c_2X^2 + c_3X^3 + c_4X^4 \quad \text{..... (3)}$$

where

RH = Relative Hardness [Ft/Fo]

t = Cooking time, min

Ft = Mean peak compression force at any time t, N

Fo = Mean peak compression force of uncooked (raw) vegetables, N

Table 2 shows the coefficients of empirical equations 1,2 and 3. To reduce the effect of absolute values of the mean peak compression force, in the empirical equation, they were converted into dimensionless numbers and were denoted as relative hardness. It is also reported that the relative hardness correlates better with the degree of cooking than the absolute value of F, as they denote the change in texture from a specific initial value (Kozempel 1988). Hardness was associated with the maximum compression force 'F' and expressed relative to time '0' as relative hardness F/Fo (compression force at any time/compression force at time 0). The empirical equations were obtained as discussed earlier.

Finally, the degree of cooking was correlated with relative hardness. The degree of cooking was derived from the cooking times. The initial value of cooking time (raw vegetable) was taken as 0% cooking and the terminal point of cooking time as 100% cooking. The terminal point (completely cooked) was decided by the point of cooking after which, there was no appreciable change in the peak compression force. This is in accordance with the studies by Tijskens and Schijvens (1987). They have reported that for longer processing times, the texture of vegetables reaches a constant level.

TABLE 2. CONSTANTS IN EQUATIONS 1, 2 AND 3

	Green peas	Carrot	Knol-khol	Radish
a_0	83.0000	94.7200	71.2500	33.7600
a_1	-8.1100	-10.8650	-11.7680	-1.6890
a_2	0.3610	0.5330	1.0950	0.0340
a_3	-0.0055	-0.0089	-0.0478	-0.0002
b_0	0.9970	0.9980	1.0000	1.0000
b_1	-0.1820	-0.2200	-0.1670	-0.0439
b_2	0.0227	0.2900	0.0158	0.0057
b_3	-0.0014	-0.0019	-0.0007	------
c_0	367.8700	114.9410	118.9410	116.3420
c_1	-2807.6800	-115.0970	-0118.211	-116.0000
c_2	10466.1000	-	-	-
c_3	-16521.7000	-	-	-
c_4	8495.3700	-	-	-

The empirical equations developed were checked by repeating the experiments and predicting the corresponding values of degree of cooking at different time intervals. The data for 4 vegetables shown in Fig 1, indicate that the model predicts the degree of cooking close to the measured values in case of all the four vegetables investigated. However, little deviation is found in the case of green peas in the initial stages of cooking and in case of radish at the final stages of cooking. This may be explained by the shape of green peas and variation in sizes of 9.5±1 mm and the mean peak compression force determined. Also, in the case of radish, extended cooking of beyond 30 min will weaken the fibres that give strength to the structure of the vegetable (Kasai et al. 1994). Hence, the measured value of degree of cooking will drop as compared to that predicted by the model. In all the three cases of cylindrical samples, the prediction correlates well with that of the measured values of upto 30 min of cooking.

To evaluate the accuracy of the fit for each coefficient of linear regression (R^2) and the mean deviation, modulus (P) was used as the criterion as shown in the equation 4. The R^2 value and P

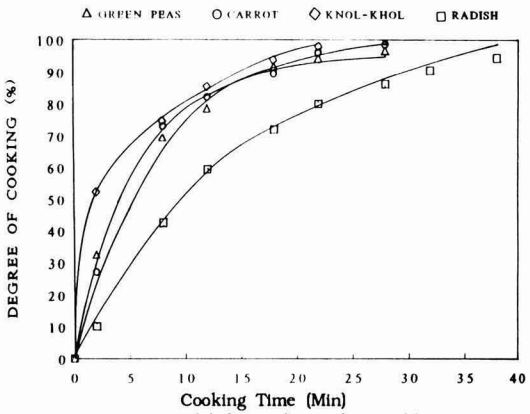

Fig. 1. Model for cooking of vegetable
Predicted (lines) and measured (symbols) values
Δ: greenpeas O: Carrot ◊: Knol-Khol □: Radish

values for 4 vegetables and for 3 equations were found to vary between 0.988 and 1, and 5 and 8, respectively. This shows the validity of the fit of predicted values with the measured values.

$$P = \frac{100}{n} \sum_{i=1}^{i=n} \frac{|(Fti_{act} - Fti_{prd})|}{Fti_{act}} \quad \ldots\ldots\ldots\ldots (4)$$

Lamauro et al (1985) have shown that a P value of less than 5 (95% confidence limit) corresponds to extremely good fit, a P value between 5 and 10 (>90% confidence) shows a reasonably good fit and a P value of above 10 (90% confidence) is considered a poor fit. Hence, knowing the cooking time, the average peak load or the relative hardness of the cooked vegetable and the degree of cooking can be obtained using the above experimental equations.

The results of the present study indicate that there is a definite relationship between mechanical properties of vegetables (green peas, carrot, knolkhol and radish), mean compression force and the cooking time. This relationship can be expressed as simple, empirical equations. The empirical formula that has been developed can be applied to determine the degree of cooking at any cooking time. This data can be used in the design of large scale cooking units and to decide the parameters to control the degree of cooking, based on the final application of the cooked product. Though water cooking was adopted in the present study, the degree of cooking and the mean peak force will not vary, if steam cooking is adopted. Hence, knowing the mean peak compression force of the cooked vegetable, the degree of cooking can be estimated, using these experimental equations.

Authors thank Sri. A. Ramesh, Head, Food Engineering Department and Dr. S.G. Prapulla and colleagues of the Bio-Engineering laboratory for providing facilities. They also thank Mr. Vatchravelu for assistance in the instrumental analysis.

References

Bera MB, Salu KL, Mukherjee B, Barale M, Sherman YK (1990) Temperature dependence of the softening and cooking rate of faba bean *dhal*. J Food Sci Technol 27:15-18

Bourne MC (1987) Effect of blanch temperature on kinetics of thermal softening of carrots and green beans. J Food Sci 52:667-668, 690

Boyd JV, Sherman P (1975) The mechanics of stickiness evaluation in foods, a comparison of oral and instrumental methods of evaluation in selected foods. Bio Rheology 12: 317-320

Huang YT, Bourne MC (1983) Kinetics of thermal softening of vegetables. J Tex Studies 14: 1-9

Kasai M, Hatae K, Shimada A (1994) A kinetic study of hardening and softening process in vegetables during cooking. J Japan Soc Food Sci Technol 41 (12): 933-941

Kostaropoulos AE (1981) The introduction of an empirical expression in the texture studies of vegetables. Food Sci Technol 14:97-99

Kozempel FM (1988) Modelling the kinetics of cooking and precooking potatoes. J Food Sci 53:753-755

Lamauro CJ, Bakshi AS (1985) Finite element analysis of moisture diffusion in stored foods. J Food Sci 50:392-396

Mittal GS (1994) Thermal softening of potatoes and carrots. Food Sci Technol 27:253-258

Sawyer FM (1971) International of sensory panel and instrumental measurement. Food Technol 25(3):51-52

Schutz HG, Marters M, Wilsher B, Rod Botten M (1974) Consumer perception of vegetable quality. Acta Hort 163:31-35

Tijskens LMM, Schijvens EPHM (1984) Preservation criteria based on texture In: Paulus KO (ed) Influence of HTST treatments on product quality and nutritive value of food and feed. Third workshop COST 91. bis, Wageningen, The Netherlands pp 84-102

Van Loey A, Francis, A Hendrickx M, Measmans G, Toback P (1994) Kinetics of quality changes of green peas and white beans during thermal processing. J Food Engg. 24:361-371

Received 1 December 1995; revised 5 November 1996; accepted 14 November 1996

J. Food Sci. Technol., 1997, Vol. 34, No. 3, 222-224

Dry-milling of Maize (*Zea Mays L.*) and Preparation of Its Fortified Products

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Studies were conducted on dry milling of maize in order to obtain the germ-free maize flour. The soaking and roasting parameters were studied for easy degerming operation. It was observed that the maize grain required 46 min at the temperature of 40°C to achieve the desired moisture content of 25%. Best roasting effect for easy degerming was achieved at 120°C and 10 min. The various products like *halwa, chapati* and *pakoda* prepared from degermed maize *sujt* and fortified flour, respectively were organoleptically well accepted.

Keywords: Dry-milling, Sensory characteristics, Shelf-life, Roasting, Degerming, Fortification.

Presently, maize is processed by dry or wet milling methods. Maize flour obtained from milling is less palatable due to the presence of tannins in the bran. If the germ portion of maize, which contains oil, is not separated from the grain, it leads to the development of rancidity, thereby reducing the shelf-life of the maize flour and its products. At present, a major lot of the maize crop is being processed into corn flakes and corn oil in large commercial mills. Utilization at small scale level for producing maize flour for making pancakes (*roti*), *pakoda, halwa* etc. is not common.

In view of this, an investigation was undertaken to adopt/develop a small scale process and technology for producing degermed maize flour. This was achieved through the conditioning and roasting of maize grains in order to enhance degerming operation and obtain maximum yield of grits, followed by grinding of these grits into flour. The maize flour, thus, obtained was further fortified with soyflour and chickpea flour for enhancing the nutritional quality of the products.

Maize (variety 'Ganga-5') grown at the experimental farm of Central Institute of Agricultural Engineering, Bhopal was used in the present experiment.

Cleaning: Maize grains were cleaned with a vibratory screen grain cleaner to remove undesirable and foreign material.

Soaking: The cleaned grains were soaked in water for swelling of endosperm and loosening of the outer husk. This further resulted in easy degerming operation and subsequent germ separation (Deshpande and Singh 1994). The soaking parameters were optimized to determine time-temperature relationship. Soaking vessels (500 ml capacity) filled with 200 ml water were placed in a water bath. After the vessel attained the predetermined bath temperatures of 40,50 and 60°C, respectively (Deshpande and Singh 1992), 400g sample was placed in these vessels and stoppered.

At specific soaking time intervals, the vessels were removed and their contents immediately transferred to a blotting paper lined cardboard box, where superficial moisture was removed by gently rolling the grains in the box (Deshpande 1990). Samples were then quickly placed in an air oven maintained at 95°C for 24h to determine the moisture content (USDA 1970).

Roasting: For optimization of roasting parameters, the grain samples were placed in a convective air oven. It had a provision of hot air circulation with the help of inside fan in order to maintain uniform temperature. The roasting was carried out at 80, 90 and 120°C for 10 min, respectively. Thereafter, the roasted grains were degermed.

Degerming: The degerming operation was performed in a pin mill having three rows of pins. For degerming, the machine was run at a speed of 2800 rpm. After the pinned rotor attained the desired speed, 5 kg roasted grains were fed in the hopper. The material passing through the outlet was collected and separated in a specific gravity separator into different fractions, viz, grits, germ, bran, powder and whole kernels. Degerming was effectively carried out, using vertical stone burr mill used for splitting/grinding of granular agricultural materials. The mill consisted of emery carborandum stones of 245 mm dia and 60 mm thickness with adjustable clearance between the stones.

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Milling of grits: The maize grits were fed in the burr mill for grinding. The maize flour obtained was passed through IS: 120 sieve. The *suji* was retained on IS:70 sieve, whereas flour was retained on IS:120 sieve.

Fortification of maize flour with soy/chickpea flour: To enhance the nutritional quality and increase the protein content, the degermed maize flour was blended with full fat soyflour and chickpea flour. The blending proportion for maize flour: chickpea flour: soyflour was maintained at 75:15:10 (Gandhi et al. 1983). The fortified maize flour was used in preparing pancake and *pakoda*.

Sensory evaluation: Sensory evaluation was conducted on different recipes like pancake, *pakoda* and *halwa* by a panel of 9 trained judges, who included housewives also. The various characteristics like taste, colour, flavour, feeling, appearance and general acceptability for each of the product were assessed (IS:6273-1971), using a 9-point Hedonic scale of excellent = 9, very good=8, good=7, below good and above fair=6, Fair=5, below fair and above poor=4, poor=3, very poor=2 and extremely poor=1.

Analysis of variance (ANOVA) was used to test the differences among the characteristics and panelists for the various products (IS:6273-1975).

The desired moisture content according to earlier investigators for easy degerming of maize and subsequent processing operations is 25% (Deshpande and Singh 1994; Brekke et al. 1971). The time intervals required to achieve this moisture content were observed to be about 46 and 20 min, respectively at the soaking temperature of 40, 50 and 60°C.

The roasting studies revealed that the roasting temperature affected degerming operation (Table 1). Maize grains were roasted for different temperatures (i.e., 70 to 130°C) and time intervals (i.e.) 1 to 10 min) in order to select best roasting treatment. The grains roasted at 120°C for 10 min gave better degerming efficiency (i.e., about 89%), whereas roasting below or above 120°C gave more breakage of grits into powder and there was not significant increase in the degerming efficiency.

The roasted grains degermed in burr mill gave better results in terms of germ separation, grits recovery and less whole (i.e., without degerming) grains. The grain roasted at 120°C for 10 min and subsequently, degermed in burr mill gave 9.3% germ separation (of available), 80% grits recovery, 4.2% (of available) bran separation and only 6.5% whole grain (without degerming).

TABLE 1. MASS FRACTION OF THE MATERIAL OBTAINED FROM PASSING THROUGH BURR MILL AND PIN MILL AT DIFFERENT ROASTING TEMPERATURES AND TIME (10 MIN).

Roasting temperature, °C	Whole maize, %	Grits, %	Germ, %	Bran, %
		Burr mill		
80	15.5	72.2	8.1	4.3
90	11.6	75.8	8.6	4.0
120	6.5	80.0	9.3	4.2
		Pin mill		
80	35.3	56.7	4.7	3.3
90	30.0	60.5	6.5	3.0
120	18.1	71.0	8.8	3.1

Theoretical germ level was 12.1% of the grain

Sensory evaluation of fortified products: The products were organoleptically evaluated and the scores awarded were computed. The mean score values for all the quality characters, including general acceptability are given in Table 2. The scores were allotted as compared to original recipes without maize flour. The mean scores for all the quality characters were more than the minimum acceptable score of 6, thereby showing their preference by the panelists.

Analysis of variance was done for each character such as taste, flavour etc. from every individual score of taste panel for different products. The results are presented in Table 3. The results revealed no significant difference at 5% level for all characteristics, except taste and feeling. Further, the disagreement among the judges for all the characteristics was not found to be significant at 5% level.

Thus, the data have indicated that the source of variance among the products is due to only two quality characteristics i.e., taste and feeling. This is obvious due to the inhibitory attitude of people towards soyflour blended maize flour, because of its inherent characteristic qualities (beany flavour). It is also expected, as it is a new product and different from the conventional foods.

TABLE 2. MEAN SCORES FOR SENSORY CHARACTERISTICS OF DIFFERENT MAIZE PRODUCTS

Characteristics	Products		
	Pancake	*Pakoda*	*Halwa*
Taste	8.11	8.29	9.32
Flavour	8.03	7.61	8.50
Colour	7.51	7.73	8.10
Feeling	7.78	7.91	7.95
Appearance	7.04	7.21	8.20
General acceptability	7.70	7.85	9.13

TABLE 3. ANALYSIS OF VARIANCE OF THE SENSORY SCORES OF DIFFERENT PRODUCTS

Source of variation	Degrees of freedom	Mean sum of squares					
		Taste	Flavour	Colour	Feeling	Appearance	General acceptability
Product	2	9.57**	1.60*	1.67*	11.93**	1.22*	1.80*
Judges	8	0.11*	0.42*	0.34*	0.91*	0.41*	0.70*
Error	16	0.18	0.31	0.29	0.04	0.36	0.27

* Not significant at 5% level

** Significant at 5% level

References

Brekke OL, Griffin Jr EL, Brooks P (1971) Dry milling of opaque-2 corn. Cereal Chem 48: 499-511

Deshpande SD (1990) Studies on some engineering aspects for processing and utilization of soybean. Ph.D. Thesis, Indian Institute of Technology, Kharagpur, India

Deshpande SD, Singh KK (1992) Hydration characteristics of maize (*Zea Mays L.*). In: Proceedings International Agricultural Engineering Conference, Bangkok, Thailand, 7-10 December, 1992 pp 601-606

Deshpande SD, Singh KK (1994) Development of technology for dry milling of maize for the production of flour and its fortified products at small scale level. Final Report on the Project No. P1-91/17-IAE-N20, CIAE/ICAR New Delhi

Gandhi AP, Mishra VK, Ali N (1983) Organoleptic assessment of full fat soyflour in various indigenous products. J Food Technol 18:771-775

IS:6273 (1971) Guide for Sensory Evaluation of Foods, Part II. Bureau of Indian Standards, Manak Bhawan, New Delhi

IS:6273 (1975) Guide for Sensory Evaluation of Foods, Part III. Statistial Analysis of Data. Bureau of Indian Standards, Manak Bhawan, New Delhi

USDA (1970) Official Grain Standards of the United States, US Dept of Agricultural Consumer and Marketing Service, Grain Division, Washington DC

Received 30 January 1995; revised 9 January 1997; accepted 13 January 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 3, 225-227

Effect of Processing and Storage on the Bacterial Quality of Edible Oyster *Crassostrea madrasensis* (Preston)

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Bacterial quality of farm grown oyster *Crassostrea madrasensis* at harvest and after processing by various methods such as depuration, shucking, antioxidant treatments, freezing and frozen storage at -18°C was studied. Depuration of oyster resulted in cleansing to an acceptable level of faecal coliforms. Pathogens like motile Aeromonads, Staphylococci, Vibrios and Salmonellae were persistant even after 24 h of depuration. The process of shucking oyster meat manually after heat treatment increased the total plate counts by 12-folds. Freezing and frozen storage decreased the bacterial counts in both antioxidant-treated and untreated samples. Motile Aeromonads, although decreased after freezing, remained viable alongwith vibrios and Staphylococci in oyster meat even after 5 months of storage at -18°C.

Keywords: Edible oyster, *Crassostrea madrasensis*, Bacterial quality, Depuration, Shucking, Freezing.

Until recently, oyster meat was used to a very limited scale for human consumption (Franssu 1988; Jasmine et al. 1991). Due to the grave risk factor associated with filter feeding capacity of oysters, the growing, harvesting and marketing of this bivalve species requires some rather special precautions in order to adequately protect the consumers from health hazard from the point of view of microbial pathogens (Richards 1988). It has been reported that the shelf-life and bacterial quality of bivalves vary according to species, bacterial quality of growing water areas, processing controls, refrigeration and other conditions (APHA 1976). The processing of oysters for human consumption either by freezing or canning involves operations such as depuration, shucking, blanching, etc. Except depuration (Richards 1988), reports on the microbial quality of oysters at various stages of processing are limited. The present communication describes the effect of processing methods on the bacterial quality of farm grown edible oyster.

Live edible oyster, *Crassostrea madrasensis* (9.96±1.35 cm in length and 6.20±1.27 cm in breadth) grown at Central Marine Fisheries Research Institute, Tuticorin Regional Research Farm by rack culture technique in shallow bay were collected. The processes of depuration, shucking, antioxidant treatments, freezing and frozen storage of oyster used were as detailed in earlier reports (Abraham et al. 1994; Balasundari et al. 1994). The oysters, freshly harvested, depurated, shucked, treated with antioxidants [clove powder (0.2% w/w) and butylated hydroxy toluene (BHT, 0.02% w/w)], frozen and frozen stored at -18°C for 5 months were analysed for the changes in total plate counts (TPC), total coliforms (TC), faecal coliforms (FC), *Enterococcus faecalis* counts (EFC), staphylococcal counts (SC), motile Aeromonad counts (MAC) and the presence of human pathogens such as vibrios and salmonellae. APHA (1976) methods were followed for the preparation of samples, enumeration of microorganisms and isolation and identification of pathogens. MACs were determined by the method of Palumbo et al (1985).

The results of the bacterial quality of oysters

TABLE 1. EFFECT OF PROCESSING AND STORAGE ON THE BACTERIAL QUALITY OF EDIBLE OYSTER MEAT

Treatments	Log counts/g					
	Total plate count	Staphylococci	Enterococcus faecalis	Motile aeromonads	MPN Total coliforms	MPN Faecal coliforms
Fresh	3.26	< 0.30	1.93	0.70	1.24	0.90
Depurated	4.23	< 0.30	<0.30	0.70	0.63	0
Shucked	5.34	2.61	0.60	1.40	1.57	1.34
Freshly frozen						
BHT-treated	4.48	2.55	0.30	0.90	0.72	0.72
Clove-treated	4.54	2.41	<0.30	1.30	1.34	1.34
Untreated	4.35	2.04	<0.30	1.30	1.34	1.34
Frozen stored at -18°C for 5 months						
BHT-treated	3.41	1.60	<0.30	0.30	0	0
Clove-treated	3.45	1.87	0.78	0.60	0	0
Untreated	3.87	2.14	0.30	0.60	0	0

BHT : Butylated Hydroxy Toluene

MPN : Most Probable Number

Vibrios were encountered in all samples tested

Salmonellae were isolated only in freshly harvested and depurated oysters

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and the effect of processing methods on the bacterial populations are shown in Table 1. Oysters were found to harbour potential human pathogens and organisms of faecal origin at levels higher than recommended (APHA 1976). The levels of TPC, TC and FC observed in this study are comparable to those reported by Durairaj et al (1983). Cleansing to an acceptable level of FC (<2.3/g) was achieved within 24 h of depuration, which accounted for >96.25% reduction over the initial level, as also observed in the Philippines oysters (Palpal-Latoc et al. 1986). The EFCs were also reduced by >95.29% in 24 h. On the other hand, the TPC showed about 10-fold increase from the initial level (Table 1). Earlier studies also demonstrated an increase in TPC in the depurated oysters (Souness and Fleet 1979; Palpal-Latoc et al. 1986) due to recontamination, while in the depuration tank. The pathogens like motile Aeromonads, Staphylococci, Vibrios and salmonellae remained persistantly even after 24h of depuration. These results corroborate the earlier observations (Greenberg et al. 1982; Rowse and Fleet 1982).

The hand picking of oyster meat after heat treatment and its holding in iced condition prior to freezing increased the TPC by >12-folds. Similarly, TC, FC, EFC, SC and MAC also showed increases over depurated meat possibly due to cross contamination of the product at the time of shucking. Vibrios were also encountered in shucked meat. Freezing reduced the TPCs by 6.2-9.7 folds in both the antioxidants-treated and untreated samples. The levels of TC, EFC, SC and MAC were slightly declined after freezing, while those of FC remained constant irrespective of treatments (except in BHT treatment), as compared to shucked oyster meat. No Salmonellae were encountered in shucked, freshly frozen and frozen stored samples.

The TPCs of antioxidant-treated samples were reduced by more than one log unit over 5 months of frozen storage. The reduction was only 0.48 log unit in untreated samples. Such microbial reduction in frozen samples together with antioxidants would bring about reduced microbial activity and oxidative changes and ultimately increased shelf-life of the product. Both TC and FC became undetectable in frozen samples due to cold shock. *E. faecalis*, however, showed more resistance to freezing and frozen storage than FC. These results are in agreement with Matches and Abeyta (1986). There have been very few studies on the stability and survival of motile Aeromonads during freezing and frozen storage (Llobrera et al. 1986; Palumbo and Buchanan 1988). Although the process of freezing decreased the MACs, the Aeromonads remained viable in oyster meat along with Vibrios and Staphylococci even at the end of storage period at −18°C (Table 1). In a study on the effect of processing and storage of oyster meat, Hood et al (1984) also observed no significant changes in the number of motile Aeromonads and vibrios.

Microbiological criteria for edible oyster at the wholesale level have been set at a FC density of <230/100g and a TPC of < 5.0 X 10^5/g (APHA 1976). The results of TPC reported in the present study conform to the above criteria in all respects. However, the most probable number of FC far exceeded the limits in shucked and freshly frozen oyster meat samples. The cross contamination of oyster especially at the time of shucking can cause problems of outgrowth of pathogens and public health hazard. Therefore, considerable stress has to be laid to environmental hygiene and sanitation measures.

The authors thank the Dean, Fisheries College and Research Institute, Tuticorin for keen interest and encouragement.

References

Abraham TJ, Balasundari S, Jasmine IG, Jeyachandran P (1994) Influence of antioxidants on the sensory quality and oxidative rancidity of frozen edible oyster. J Food Sci Technol 31:168-170

APHA (1976) In : Speck ML (ed). Compendium of Methods for the Microbiological Examination of Food. American Public Health Association, New York

Balasundari S, Abraham TJ, Shanmugam SA, Jeyachandran P (1994) Depuration of edible oyster *Crassostrea madrasensis* (Preston). Poll Res 13:153-160

Durairaj S, Chinnasamy G, Mohamed MS (1983) A bacteriological study of the natural flora of edible oyster, *Crassostrea madrasensis*. Fish Technol 20:111-114

Franssu LD (1988) Oysters. INFOFISH Intl 3:14-16

Greenberg EP, Dubois M, Palhof B (1982) The survival of marine vibrio in *Mercenaria mercenaria*, the hard shell clam. J Food Safety 4:113-123

Hood MA, Baker RM, Singleton FL (1984) Effect of processing and storing oyster meats on concentrations of indicator bacteria, vibrios and *Aeromonas hydrophila*. J Food Prot 47:598-601

Jasmine IG, Rajagopalasamy CBT, Sugumar G, Jeyachandran P (1991) Quality characteristics of freeze dried edible oyster *Crassostrea madrasensis* (Preston). J Food Sci Technol 27:392-393

Llobrera AT, Bulalacao ML, Sunaz N (1986) Effects of storage on the microbial quality of slipper oysters, *Crassostrea iredalei*. In:Maclean JL, Dizon LB, Hosillos LV (eds). Proceedings of the First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines, pp 437-442

Matches JR, Abeyta C (1986) Indicator organisms in fish and shellfish. Food Tech 37:114-117

Palpal-Latoc EQ, Garcia RP, Caoile SJS, Luna CZ, Cariaga AM, Fernandez SA (1986) The sanitary quality of four Philippines oyster growing areas. In: Maclean JL, Dizon LB, Hosillos LV (eds). Proceedings of the First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines, pp 447-450

Palumbo SA, Buchanan RL (1988) Factors affecting growth or survival of *Aeromonas hydrophila* in foods. J Food Safety 9:37-51

Palumbo SA, Mayino F, Williams AC, Buchanan RL, Thayer DW (1985) Starch ampicillin agar for the quantitative detection of *Aeromonas hydrophila*. Appl Environ Microbiol 50:1027-1030

Richards GP (1988) Microbial purification of shellfish: A review of depuration and relaying. J Food Prot 51:218-251

Rowse AJ, Fleet GH (1982) Variability and release of *Salmonella charity* and *Escherichia coli* from oyster faeces. Appl Environ Microbiol 44:544-548

Souness RA, Fleet GH (1979) Depuration of Sydney rock oyster, *Crassostrea commercialis*. Food Technol Aust 31:397-404

Received 2 December 1994; revised 25 January 1997; accepted 27 January 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 3, 228-229

Prevalence of Motile Aeromonads in Foods of Animal Origin

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Motile aeromonads were isolated from 125 of 507 (24.6%) fresh food samples of sheep and poultry origin. The distribution of these organisms was 37.5% in sheep meat, 32.6% in edible organs of poultry, 28% in poultry meat, 24.2% in edible organs of sheep and 0.9% in poultry eggs. The isolated strains were identified and differentiated into 77 *A. hydrophila*, 43 *A. caviae* and 5 *A. sobria*. Though enterotoxin and haemolysin were produced by the strains of all the three species of *Aeromonas*, it was more common in *A. hydrophila* and *A. sobria* strains than the *A. caviae* strains.

Keywords: Aeromonads, Poultry meat, Sheep meat, Eggs, Enterotoxin, Haemolysin.

Motile aeromonads have been implicated as causative agents of human gastroenteritis (Gracey et al. 1982) and other ailments such as meningitis, endocarditis and osteomyelitis in humans (Davis et al. 1978; Ellison and Mostow 1984; Karam et al. 1983). These organisms occur widely in the environment, especially in water (Hazen et al. 1978) and have been isolated from a wide range of foods (Palumbo et al. 1989). This has raised the possibility that they may be a potential cause of food-associated ailments. *Aeromonas* associated food-borne outbreaks and a number of sporadic cases have been reported from abroad (Agbonlahor et al. 1982; Abeyta et al. 1986). However, no systematic studies appear to have been conducted to determine the occurrence of these organisms in various foods in India. Therefore, the present investigation was aimed to study the prevalence of motile *Aeromonas* spp. in retail poultry and sheep meat including their edible organs and poultry eggs and to test their ability to produce haemolysin and enterotoxin.

Five hundred and seven samples comprising poultry eggs (102), poultry meat (100), edible organs (liver and heart) of poultry (101), sheep meat (102) and edible organs (liver and kidney) of sheep (102) were collected from local markets and different poultry farms in Haryana during the period from October 1991–March, 1992. The samples were collected in polythene bags and were transported to laboratory in ice box. The samples were examined on the day of their collection.

Isolation of aeromonads from collected samples was done as per the procedure of Majeed et al (1989). Alkaline peptone water (APW) as enrichment and starch ampicillin agar (SAA) as selective medium were prepared, as described by Shread et al (1981) and Palumbo et al (1985), respectively. Twenty ml each of the inoculum of egg shell and egg yolk as per the procedure followed by Khurana and Kumar (1994), 20g each of minced meat of poultry and sheep was mixed separately with 180ml of APW in 250 ml conical flasks, which were incubated at 28°C for 24h. These cultures were streaked on SAA and presence of large (3–5mm), honey yellow, amylase positive colonies (those having a clear zone surrounding the colony after flooding with iodine solution) were considered as presumptive aeromonads.

The purified presumptive aeromonads were identified to the genus level according to the method of Majeed et al (1989) and were differentiated to species level by the biochemical tests including five differential key tests as proposed by Popoff (1984). These tests were indole production, Voges Proskauer test, acid from arabinose, salicin, sucrose and mannitol, hydrolysis of aesculin, gas from glucose, H_2S from cysteine, breakdown of inositol and utilization of L-arginine.

The representative isolates of *Aeromonas* (27) isolated from different foods were tested for their ability to produce enterotoxin and hemolysin using suckling mouse technique, as described by Megraud (1986) and haemolysin activity was assayed with rabbit erythrocytes (Burke et al. 1981). Cell-free supernatant fluids were prepared as per the method described by Majeed et al (1989). In the suckling mouse technique, the ratio (combined intestinal wt to combined remaining body wt) of 0.08 was considered as positive and the filtrates causing lysis in 50% of erythrocytes by visual inspection were scored as haemolytic. Results were expressed as

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TABLE 1. DISTRIBUTION OF MOTILE AEROMONADS IN MEAT AND EGGS

Sample type	Samples examined	Number of strains	Aeromonas species		
			A. hydrophila	*A. sobria*	*A. Caviae*
Poultry meat	100	28	15	ND	13
Liver and heart (Poultry)	101	33	27	ND	6
Sheep meat	102	38	19	5	14
Liver and kidney (Sheep)	102	25	15	ND	10
Eggs : Yolk	102	1	1	ND	ND
Shell		ND	ND	ND	ND
Total	507	125	77	5	43

the reciprocal of the greatest dilution showing haemolysis.

In the present investigation, 125 strains of motile *Aeromonas* were isolated from 507 samples (24.6%) of meat and eggs, using combination of APW and SAA techniques. Majeed et al (1989) have also recommended uses of enrichment broth and selective medium for isolation of *Aeromonas*.

Out of these 125 isolated strains, 77 (61.6%) were identified as *A. hydrophila*, 43 (34.3%) *A. caviae* and 5(4.0%) *A. sobria* (Table 1). The isolation rate of these microorganisms from meat and edible organs of sheep and poultry varied from 24.5 to 37.5. The high isolation rate from meat and organs confirms the earlier findings of Palumbo et al (1985) and Majeed et al (1989) that these organisms are ubiquitous in meat. Only one out of 102 (0.98%) samples of egg yolk yielded *A. hydrophila*, while the remaining eggs were free of contamination. The isolation of *Aeromonas* from egg contents has earlier been reported by Board et al (1964).

Twenty seven representative strains of *Aeromonas* comprising 14 of *A. hydrophila*, 11 of *A. caviae* and 2 of *A. sobria* were tested for enterotoxin and haemolysin production. Six (22.2%) of the strains produced both enterotoxin and haemolysin simultaneously, 12 (44.4%) produced enterotoxin alone and 11 (40.7%) haemolysin alone. The remaining 10 (37.4%) strains were negative for both the factors. The data showed that greater number of *A. sobria* (100% and 50%) and *A. hydrophila* (57.1% and 42.8%) produced enterotoxin and haemolysin than *A. caviae* (18.1% and 36.3%). These results confirm the earlier observation of Majeed et al (1989).

It may be concluded from this study, poultry and sheep meat including their edible organs have high carriage rate of motile aeromonads, which may possibly be involved in the transmission of this infection to human beings. Because of virulent nature of isolated strains of *Aeromonas*, the infected foods may be responsible for human gastroenteritis.

References

Abeyta C, Kaysner CA, Wekell MM, Sullivan JJ, Stelma GN (1986) Recovery of *Aeromonas hydrophila* from oysters implicated in an outbreak of foodborne illness. J Food Pro 49:643-646

Agbonlahor DE, Shonekan RA, Kazak WH, Coker AO (1982) *Aeromonas* food poisoning in Nigeria: a case report. Central Afr J Med 28:36-38

Board RG, Ayres JC, Kraft AA, Forsythe RH (1964) The microbiological contamination of egg shells and egg packing materials. Poult Sci 43:584-595

Burke V, Robinson J, Atkinson HM, Dibley M, Berry RJ, Gracey M (1981) Exotoxins of *Aeromonas hydrophila*. Aust J Exp Biol Med Sci 59:753-761

Davis WA, Kane JG, Garagusi VF (1978) Human *Aeromonas* spp. infections: A review of literature and a case report of endocarditis. Medicine 57:267-277

Ellison RT, Mostow SR (1984) Pyogenic meningitis manifesting during therapy for *Aeromonas hydrophila* sepsis. Arch Intern Med 144:2078-2079

Gracey M, Burke V, Robinson JR (1982) *Aeromonas*-associated gastroenteritis. Lancet ii:1304-1306

Hazen TC, Fliermans CB, Hirsch RP, Esch GW (1978) Prevalence and distribution of *Aeromonas hydrophila* in the United States. Appl Environ Microbiol 36:731-738

Karam GH, Ackley AM, Dismukes WE (1983) Post-traumatic *Aeromonas hydrophila* osteomyelitis. Arch Intern Med 143:2073-2074

Khurana Rajesh, Kumar Ashwani (1994) Studies on bacterial load of eggs and meat. Indian J Vet Res 3:25-30

Majeed K, Agan A, Macrae IC (1989) Enterotoxigenic aeromonads on retail lamb meat and offal. J Appl Bacteriol 67:165-170

Megraud F (1986) Incidence and virulence of *Aeromonas* species in faeces of children with diarrhoea. Eur J Clin Microbiol 5:311-316

Palumbo SA, Bencivengo MM, Correl FD, Williams AC, Buchanan RL (1989) Characterization of the *Aeromonas hydrophila* group isolated from retail foods of animal origion. J Clin Microbiol 27:854-859

Palumbo SA, Maxino F, Williams AC, Buchanan RL, Thayer DW (1985) Starch ampicillin agar for the quantitative detection of *Aeromonas hydrophila*. Appl Environ Microbiol 50:1027-1030

Popoff M (1984) *Aeromonas*. In: Kreig NR (ed) Bergy's Manual of Systematic Bacteriology. Williams and Wilkins, Baltimore, pp 545-548

Shread P, Donovan TJ, Lee JV (1981) A survey of the incidence of *Aeromonas* in human faeces. Soc Gen Microbiol 8:184-186

Received 16 March 1995; revised 27 November 1996; accepted 9 December 1996

J. Food Sci. Technol., 1997, Vol. 34, No. 3, 230-232

A Model for Osmotic Concentration of Banana Slices

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The effect of temperature and initial sugar syrup concentration on osmotic concentration of banana slices were studied at an initial ratio of banana slices: sugar solution as 1:4. The water loss varied with sugar concentration as well as temperature. The mass transfer coefficient was found to increase with sugar concentration and temperature of the solution. The developed model can be used for prediction of water loss during osmotic concentration of banana slices within the range of experimental study.

Keywords: Osmotic concentration, Diffusion kinetics, Banana.

Osmotic concentration is the process of water removal by immersing in a water-containing cellular solid in a concentrated aqueous sucrose solution. The main advantages of the process are: inhibition of enzymatic browning, retention of natural colour without addition of sulphites, high retention of volatile compounds during subsequent drying and low energy consumption.

Osmotic concentration as an intermediate step in air or vaccum drying of fruits and vegetables was studied by Kim and Taledo (1987). Kinetics of dewatering and mass transfer properties during the osmotic process have been investigated for apple (Ponting et al. 1966; Hawkes and Flink 1978; Conway et al. 1983). Banana is a major fruit in many countries and for extension of shelf life, the osmotic concentration technique could be applied for banana dehydration as well.

The objectives of the present study were to study the effect of temperature and sugar syrup concentration on moisture diffusion and to model the kinetics of osmotic concentration of banana slices.

Fresh ripe bananas were peeled, cut into slices of 0.5 cm thickness and 2.5 cm dia, washed and treated with 0.25% sodium metabisulphite solution for 15 min. The experiments were carried out at 3 different concentrations (50, 60 and 70° Brix) and three temperatures (30, 40 and 50°C), maintaining a solid to liquid ratio of 1:4.

One banana slice each was placed in glass beakers containing the sugar solution. A shaking water bath was used to maintain a constant temperature of the sugar solution. For every 30 min, one glass beaker was removed from the water bath and the banana slice was immediately rinsed with water and surface-dried. The slice was weighed and its moisture content was determined in a vacuum oven (AOAC 1984).

To analyse the data, the water loss was calculated. The water loss (WL) was the net loss of water from banana slice at time 'θ' on an initial weight basis.

$$WL = \frac{Wsi\ Xswi - Ws\theta\ Xsw\theta}{Wsi} \times 100 \quad ....(1)$$

Where,

WL= Water loss (g water/100g initial banana slice)

Wsi= Initial weight of banana slice, of

Wsθ= Weight of slice after time 'θ' of

Xswi= Water content as a fraction of the initial weight of the slice.

Xswθ= Water content as a fraction of the weight at time 'θ'.

The model was verified by using osmotic concentration unit under 70° Brix, sugar solution (B), 50°C temperature (T) and 30 min interval contact time (θ).

Banana slices (500g) were flatly laid out on a stainless steel wire mesh, cage housing. The whole cage assembly was put into osmotic chamber holding 2000g sugar solution. The solution temperature was maintained (50°C) with the help of thermostat. The solution was gently circulated by a centrifugal pump. After a designated period of time, the sample was drawn out, surface-dried and their moisture content was determined.

The kinetics of osmosis of all the experiments are shown in Fig. 1, 2 and 3. The water loss increased with increase in sugar syrup concentration and temperature. The water loss was very fast at the beginning of the process, decreasing gradu-

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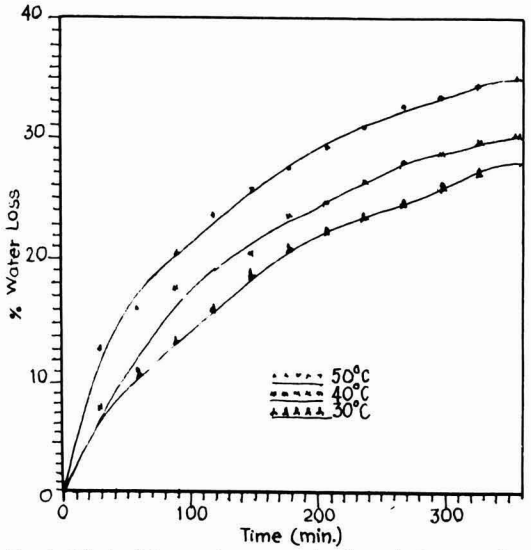

Fig. 1. Effect of temperature on water loss during osmotic concentration at 50° Brix

ally with length of contact, approaching to equilibrium.

It was observed that the water loss from the banana slice, concentrated at 50° Brix and 50°C was similar to the water loss from the slice concentrate at 40°C, 60° Brix and 30°C, 70° Brix. It was concluded that the every 10°C increase in temperature or by increasing in 10° Brix of sugar solution, there was an almost equal increase in the final water loss.

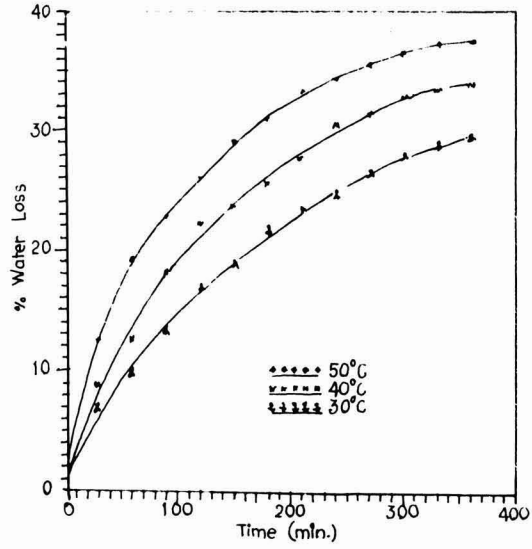

Fig. 2. Effect of temperature on water loss during osmotic concentration at 60° Brix

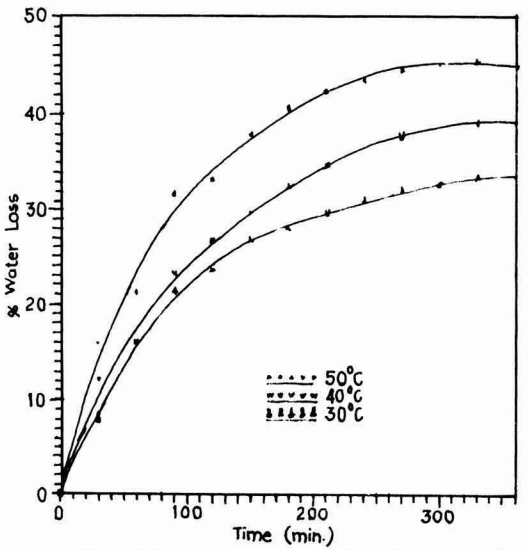

Fig. 3. Effect of temperature on water loss during osmotic concentration at 70° Brix

The resistance to water movement from banana slices to sugar solution lies in the solids. The water loss (WL) was modelled on the principles of diffusion in solids. The banana slices were soaked in limited volume of external sugar solution, with a solid: liquid ratio of 1:4. The water loss (WL), essentially a concentration parameter, was proportional to the square root of the time of contact, in min (Crank 1975; Hawkes and Flink 1978). Therefore, it could be written as

$$WL = Kw\ \theta^{1/2} \quad \text{..... (2)}$$

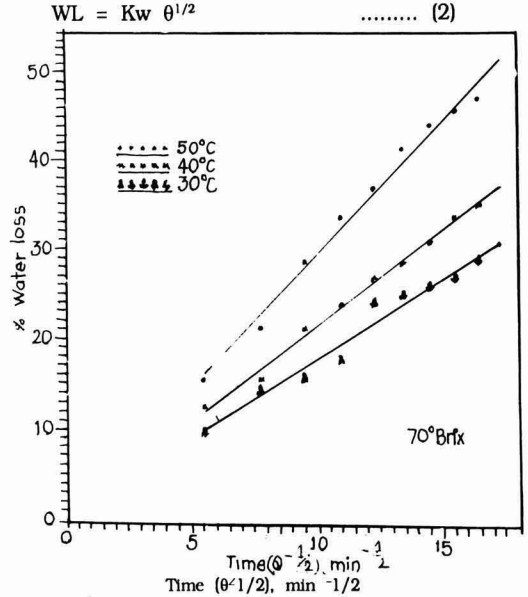

Fig. 4. Typical relationship of % water loss from banana slices to square root of contact time

TABLE 1. VARIATION OF MASS TRANSFER COEFFICIENT (KW) WITH SUGAR SYRUP CONCENTRATION AND TEMPERATURE

Sugar syrup concentration, °Brix	Sugar solution temperature, °C	Mass transfer coefficient, Kw	
		Experimental	Predicted
50	30	1.567	1.455
50	40	1.781	1.757
50	50	1.992	2.021
60	30	1.617	1.642
60	40	1.884	2.005
60	50	2.284	2.333
70	30	1.795	1.843
70	40	2.218	2.242
70	50	2.921	2.891

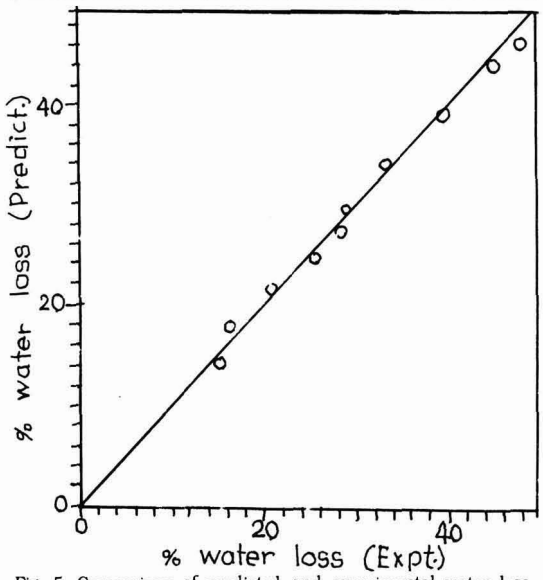

Fig. 5. Comparison of predicted and experimental water loss from banana slices

Where, Kw is mass transfer coefficient expressed as $(min)^{-1/2}$

The values of mass transfer coefficient, (Kw) were obtained from the slope of the straight line plots between water loss and time $\theta^{1/2}$ (Fig.4). The mass transfer coefficient, (Kw) is a function of sugar syrup concentration, °Brix (B) and temperature °C (T).

The following model, similar to that of Magee et al (1973) is proposed for Kw.

$$Kw = a\ B^x\ T^y \quad \text{..... (3)}$$

Where,

B= Initial sugar syrup concentration, °Brix

T= Temperature of sugar syrup, °C

The parameters a, x and y were unique to the system and should be estimated from experimental data, as obtained through a non-linear regression, $a = 8.3224 \times 10^{-3}$, x = 0.7265 and y= 0.6804 with and r=0.94. The final equation for the water loss (WL), therefore, was :

$$WL = 8.3224 \times 10^{-3}\ B^{0.7265}\ T^{0.6804}\ \theta^{1/2} \quad \text{....(4)}$$

Table 1 and equation 4 reveal that the mass transfer coefficient, (Kw) increased with increase in sugar syrup concentration and temperature.

For testing the adequacy of the model, the values of input variables (B=70°Brix, T=50°C and θ=30 min interval) were tested experimentally. The experimental and predicted values of water loss (WL) matched very well (Fig.5). From the practical application point of view, the water loss (WL) at time (θ) may be predicted from equation 4.

References

AOAC (1984) Official Method of Analysis. Association of Official Analytical Chemists, Washington, DC

Conway J, Castaigne F, Picard G, Vavan X (1983) Mass transfer considerations in the osmotic dehydration of apples. Can Inst Food Sci Technol J 16 (1):25-29

Crank J (1975) The Mathematics of Diffusion, Clarendon Press, Oxford

Hawkes J, Flink JM (1978) Osmotic concentration of fruit slices prior to freeze dehydration. J Food Process Preserv 2:265-284

Kim MH, Toledo RT (1987) Effect of osmotic dehydration and high temperature fluidized bed drying on properties of dehydrated rabbiteye blueberries. J Food Sci 52:980-985

Magee TRA, Hassaballah AA, Murphy WR (1983) Internal mass transfer during osmotic dehydration of apple slices in sugar solution. Irish J Food Sci Technol 7(2):147-153

Ponting JD Watters GG, Forrey RR, Jackson R, Stanley WL (1966) Osmotic dehydration of fruit. Food Technol 20:125-128

Received 3 October 1994; revised 23 January 1997; accepted 25 January 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 3, 233-236

Effect of Storage and Chemical Treatment on Cooking Time and Water Absorption of Chickpea (*Cicer arietinum* L.)

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Changes in cooking time and water absorption were assessed during storage and chemically-treated chickpea seeds. Seeds of both *desi* and *kabuli* types were found prone to hard-to-cook defect. Cooking time increased significantly during storage. *Kabuli* varieties had higher cooking time than *desi*. Water absorption increased with increase in soaking period. Stored *kabuli* seeds absorbed less water, while *desi* stored seeds absorbed more water as compared to fresh ones. Significant increase in cooking time was also observed in chemically-hardened seeds. Chemically-hardened chickpeas also exhibited increase in water absorption with increase in soaking period. Both cooking time and water absorption of *desi* and *kabuli* chickpeas can be affected by storage and chemical treatment. Chemical treatment can be used for rapid screening of chickpeas in fresh state that are susceptible to development of hard-to-cook defect during storage.

Keywords: Cooking time, Water absorption, Hard-to-cook tendency, Chickpea, Storage, Chemically-induced hardness.

Pulses are rich in proteins and constitute an important item of the diets of the population of Asia and Africa (Rao and Sastry 1991). Grain legumes are suitable protein supplements to cereals (Hulse 1991). Chickpea is the most important food legume of dry-land agriculture and mostly consumed after soaking and boiling in water to a desirable consistency along with spices and condiments. Among the cultivated species of chickpea, the two main groups of practical importance are *kabuli* gram, having large seed with thinner and light-coloured seed coat in contrast to *desi* gram , having brown seed coat and yellow endosperm.

One of the constraints associated with certain legume seeds is the existence of storage-induced hard-to-cook (HTC) defect, which is a textural defect, appearing in legume seeds stored under unfavourable conditions of high humidity and high temperature. HTC specifies acquiring of resistance in seeds to softening during cooking process, as cotyledonary cells fail to separate during cooking (Gracia and Lajolo 1994), resulting in poor cooking quality.

Storage hardening and chemical hardening are well known indices to explore HTC tendency (Liu et al. 1992; Reyes-Moreno et al. 1994). Cooking time influences consumer's preference. Development of a simple and rapid method to detect pulses susceptible to HTC defect is the demand of the time, which may also meet the future requirements of those engaged in pulse technology improvement programme. Keeping in view, the scanty and scattered information on this aspect, the present investigation was undertaken to study the effect of storage and chemical treatment on cooking time and water absorption of chickpea.

Chickpea seeds of 'Gaurav', 'C-235' and 'H 82-2' of the *desi* type and 'L-144', 'L-550' and 'Gora Hisari' of the *kabuli* type were procured from CCS Haryana Agricultural University, Hisar. Fresh seeds of the cultivars were from the 1995 harvest and stored seeds from the 1994 harvest. Recommended package of practices were followed to raise the crop under Hisar conditions (HAU 1981). The seeds were stored under identical conditions in empty godown in April, using 25 tablets (3g each) of aluminum phosphide for 100 cubic meter of empty space. Batches of 50 g fresh seeds of each variety were soaked in 50 ml of 0.1M sucrose, 0.1N calcium chloride, 0.1M acetate buffer (pH 4.0) at room temperature for 6h each and in 80% ethanol for 18h. (Jones and Boulter 1983; Liu et al. 1993; Reyes-Moreno et al. 1994). After the expiry of soaking period, soaking solution was drained and discarded. Seeds were rinsed with double distilled water, blotted dry and kept in an oven at 60°C overnight. Later, these seeds were used for cooking time and water absorption studies. Cooking time was determined using the method of William et al (1983).

Water absorption and solid loss were determined in fresh, stored and chemically-treated seeds according to the method of Hincks et al (1987).

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TABLE 1. COOKING TIME OF FRESH, STORED AND CHEMICALLY HARDENED CHICKPEA

Varieties	Fresh	Storage hardened	Sucrose	Chemically hardened chickpea		Ethanol
				Acetate buffer	Calcim chloride	
Desi						
'Gaurav'	62.0± 5.0	77.0± 0.0	73.0± 3.0	139.0± 6.0	138.0± 15.0	92.5± 2.5
'C-235'	50.5± 6.5	69.5± 6.5	75.5± 2.5	89.5± 2.5	104.0± 11.0	63.5± 1.5
'H82-2'	58.0± 0.0	62.0± 0.0	59.0± 4.0	85.0± 15.0	100.0± 0.0	62.5± 2.5
Mean	56.83	69.5	69.2	102.5	114.5	72.8
Kabuli						
'L-144'	70.0± 0.0	92.5± 2.5	150.0± 2.0	198.0± 1.0	384.0± 4.0	84.0± 4.0
'L-550'	62.0± 1.0	71.0± 2.0	245.0± 5.0	187.5± 2.5	387.5± 2.5	65.0± 0.0
'Gora Hisari'	53.5± 1.5	74.0± 0.0	81.5± 1.5	205.0± 2.5	309.5± 3.5	63.5± 1.5
Mean	61.8	79.2	162.2	196.8	353.7	70.8

C.D. at 5% Variety = 10.8

Fresh, storage hardened and chemically hardened = 10.8

Each value represents cooking time in min and average of two determinations

Samples (10g) each were immersed in 50 ml of double distilled water for 4,8, 12 and 16h at 25°C in a BOD incubator. The soaked seeds were blotted dry and weighed and the water absorption was determined. The losses of solids leached into, during steeping was taken into account and water absorption was corrected accordingly. For determining solids lost, the seed leachates were made to 50 ml volume along with the rinsed water. Twenty five ml of leachates were dried on a water bath and finally kept in oven at 80-100°C, till complete drying. The data were statistically analyzed to determine the main effects. The values are mentioned as mean ± standard error.

Data in Table 1 reveal that cooking time in both *desi* and *kabuli* chickpeas increased during storage and all *kabuli* types exhibited higher cooking time in fresh as well as in stored samples, but differences were not significant in 'L-550' and 'H 82-2' varieties. William et al (1983) have viewed that cooking time in chickpeas may be affected by the starch, the permeability of seed coat, internal structure and compactness of seed coat and endosperm material, while soaking the seeds in water. The cooking time recorded in the present study (55-200 min) are very well within the reported values. Punia and Chauhan (1993) recorded cooking time of 75-90 min in high yielding chickpeas that are also akin to the present results.

The cooking time of all chemically-treated seeds increased but to different extents as compared to control. In *kabuli* types, maximum cooking time was exhibited by calcium chloride followed by acetate buffer, sucrose and ethanol. Calcium chloride hardened 'L-550', which recorded a cooking time of 387.5 min as against 62 min in control.

TABLE 2. CORRECTED WATER ABSORPTION IN FRESH AND STORED CHICKPEAS DURING DIFFERENT SOAKING PERIODS, g 100 dry wt^{-1}

Varieties	4h		8h		12h		16h	
	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored
Desi								
'Gaurav'	20.85± 4.03	55.89±6.36	30.76± 6.23	69.49± 9.82	38.12 ±3.28	69.51± 3.50	40.39± 8.93	97.61± 1.00
'C-235'	34.29± 3.70	29.67±3.08	57.80± 0.09	68.12± 9.28	69.88 ±15.50	93.51± 0.56	72.29± 8.18	101.91± 2.41
'H82-2'	33.54± 2.50	45.08±5.60	35.40± 0.79	59.03± 10.85	45.15 ±0.72	73.79± 5.90	49.87± 5.90	89.70± 1.56
Mean	29.56	43.55	41.32	65.55	51.05	78.94	54.18	96.37
Kabuli								
'L-144'	91.07± 3.88	80.04± 1.56	106.24± 3.15	96.07± 3.03	113.44 ±0.62	102.55± 1.05	108.11± 9.20	107.78± 2.65
'L-550'	110.67± 3.44	88.88±0.70	132.42± 4.16	99.20± 0.57	134.92 ±3.42	102.30± 0.85	133.47± 3.50	102.20± 0.43
'Gora Hisari'	119.32± 11.94	106.75± 1.32	131.13± 1.51	114.45± 0.78	131.08 ±3.95	114.06± 1.08	141.22± 5.48	115.84± 3.85
Mean	107.02	91.82	123.26	103.24	126.48	106.30	127.60	108.61

C.D. at 5% Variety = 3.67

Fresh and storage = 2.11

Time factor 2.99

Each value is an average of two determinations

TABLE 3. CORRECTED WATER ABSORPTION IN FRESH AND STORED CHICKPEAS DURING DIFFERENT SOAKING PERIODS, g 100 dry wt^{-1}

Varieties	4h soaking					8h soaking					12h soaking				
	Control	Sucrose	Acetate buffer	Calcium chloride	Ethanol	Control	Sucrose	Acetate buffer	Calcium chloride	Ethanol	Control	Sucrose	Acetate buffer	Calcium chloride	Ethanol
Desi															
'Gaurav'	20.85	75.70	85.53	64.39	63.91	30.76	86.19	101.20	77.52	76.23	38.12	87.31	91.35	64.56	90.27
'C-235'	34.29	78.74	107.40	88.79	73.21	57.80	83.81	195.60	98.69	84.96	69.88	81.30	107.95	90.06	97.36
'H82-2'	33.54	75.40	97.08	54.74	84.97	35.40	82.59	96.88	68.40	86.76	45.15	82.34	101.52	73.72	81.62
Mean	29.56	76.61	96.67	69.31	74.03	41.32	84.20	101.23	81.54	82.65	51.05	83.65	100.27	76.11	89.75
Kabuli															
'L-144'	91.07	77.44	80.18	74.58	89.44	106.24	75.97	112.19	84.48	110.52	113.44	79.48	86.16	88.68	111.21
'L-550'	110.67	89.81	104.59	70.31	132.22	132.42	82.55	95.54	68.69	133.07	134.92	86,48	114.92	91.26	130.71
'Gora Hisari'	119.32	80.37	112.51	77.13	133.71	131.13	71.64	115.10	85.24	123.60	131.98	77.19	105.21	91.88	127.42
Mean	107.02	82.54	99.09	74.01	118.46	123.26	76.72	107.61	79.47	122.40	126.48	81.05	102.10	90.61	123.11

Desi chickpeas, in general, were found to be less hardened as compared to *kabuli*. Ethanol was found to be the least effective in inducing chemical hardening in both types of chickpeas. *Flor de Mayo* and *Mayocoba* types of common beans were the most and the least prone to acetate buffer hardening (Reyes-Moreno et al. 1994). Chemical hardening induced by calcium chloride in cowpea has taken place through loss of cell membrane integrity that allows cations to bind intracellular components (Liu et al. 1993). Autoradiographs of 45^{Ca} pea cotyledons showed that when seed coat intact pea cotyledons were steeped in calcium chloride solution containing labelled calcium, calcium was at the periphery of the cotyledons as against those which were soaked without seed coat, suggesting that seed coats prevented the diffusion of calcium cotyledon into the surrounding medium (Rosenbaum and Baker 1969).

Data given in Table 2 show that water absorption in *desi* and *kabuli* chickpeas, in general, increased significantly during 16h of soaking period, but maximum rapid water absorption was recorded during the first 4h and thereafter, the rate slowed down. Water absorption values decreased in *kabuli* chickpea, but increased in *desi* chickpea during storage. The differential water absorption pattern in *desi* and *kabuli* under the present experimental conditions may be due to differences in anatomical features of seed coat and testa. Moreover, water absorbing capacity depends upon cell wall structure composition of seed and compactness of the cells in seed (Muller 1967). The differential water absorption patterns were also reported earlier in pulses during storage (Burr et al. 1968; Moscosa et al. 1984; Hincks and Stanely 1986).

All chemically hardened *kabuli* chickpea seeds showed reduction in corrected water absorption at 4, 8 and 12 h of steeping as compared to control and all *desi* types showed increase in corrected water absorption (Table 3). The rapid water uptake during 4h corroborated with storage-induced hardness (Table 2). The differential deacceleration and acceleration in water absorption in these two types when steeped in acetate buffer, calcium chloride, sucrose and ethanol presently might have changed the permeability pattern of membranes to different extents that affected water uptake and information regarding hardening effect of these chemicals in pulses is already available in literature (Jones and Boulter 1983; Liu et al. 1993; Reyes-Moreno et al. 1994). The rapid water uptake in kidney bean during first 6h, after which the rate slowed down also supports the present results in chickpea (Moscoso et al. 1984). The possible reasons for reduced imbition value in ethanol-hardened soft bean cotyledons earlier reported by Jones and Boulter (1983) agree with the present results and may also be due to membrane breakdown and leakage of solutes.

The present study has shown that cooking time and water absorption in chickpea can be affected during storage and chemical treatments and chemical hardening of fresh chickpeas may be useful in future for judging hard-to-cook defect prior to storing of chickpeas.

References

Burr HK, Kon S, Morris HJ (1968) Cooking rates of dry beans influenced by moisture content, temperature and time of storage. Food Technol 22:336-338

Gracia E, Lajolo FM (1994) Starch alterations in hard-to-cook beans (*Phaseolus vulgaris*). J Agri Food Chem 42:612-615

HAU (1981) Package of Practices for Rabi Crops, Haryana Agricultural University, Hisar, India

Hincks MJ, Stanley DW (1986) Multiple mechanisms of bean hardening. J Food Technol 21:731-739

Hincks MJ, McCannel A, Stanley DW (1987) Hard-to-cook defect in black beans-Soaking and cooking processes. J Agri Food Chem 35:576-583

Hulse JH (1991) Nature, composition and utilization of grain legumesl In: Jambunathan R (ed) Uses of Tropical Grain Legumes: Proceedings of Consultant Meeting, March 27-30 1989. International Crop Research Institute for Semi-arid Tropics, Patancheru, India, pp11-30

Jones PMB, Boulter D (1983) The cause of reduced cooking rate in *Phaseolus vulgaris* followed adverse storage conditions. J Food Sci 48:623-632

Liu K, Phillips RD, McWatters KH (1993) Induced hard-to-cook state in cowpea by freeze-thawing and calcium chloride soaking. Cereal Chem 70:193-195

Liu K, Shewfelt RL, Phillips RD, Hung YC (1992) Leakage and water absorption of cowpea seeds during pre-treatment and cooking as related to hard-to-cook state. Inter J Food Sci Technol 27:683-690

Moscoso W, Bourne MC, Hood LF (1984) Relationship between the hard-to-cook phenomenon in red kidney beans and water absorption, puncture force, pectin, phytic acid and minerals. J Food Sci 49:1577-1583

Muller FM (1967) Cooking quality of pulses. J Sci Food Agric 18:292-295

Punia D, Chauhan BM (1993) Nutrient make up, level of antinutrients, cookability and consumer preferred characteristics of high yielding chickpea varieties. Bull Grain Technol 31:44-51

Rao NP, Sastry JG (1991) Legume consumption and its application on the nutritional status of population in India. In: Jambunathan R. (ed). Uses of Tropical Grain Legumes. Proceedings of Consultant Meeting. March 27-30, 1989. International Crop Research Institute for Semi-arid Tropics, Patancheru, India, pp 37-48

Reyes-Moreno C, Paredes-Lopez O, Barradas I (1994) A fast laboratory procedure to assess the hard-to-cook tendency of common bean varieties. Food Chem 49:187-190

Rosenbaum TM, Baker BE (1969) Constitution of leguminous seeds VII-Ease of cooking field peas (*Pisum sativum*) in relation to phytic acid content and calcium diffusion. J Sci Food Agric 20:709-712

William PC, Nakoul H, Singh KB (1983) Relationship between cooking time and some physical characteristics in chickpea (*Cicer arietinum*). J Sci Food Agric 34:492-496

Received 1 May 1996; revised 23 January 1997; accepted 25 January 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 3, 237-239

Studies on the Quantitative Changes of Redgram (*Cajanus cajan*) in Different Storage Conditions

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Changes in terms of weight, density and bulk density of redgram (*Cajanus cajan*) treated with protectants, like dried neem leaves (covering the top layer of sample evenly), cow dung ash (1.0%) and mustard oil (0.5%) and stored for 4 months in clay pots and tin containers were studied. After 4 months of storage, lowest values of weight, density and bulk density were recorded in cow dung ash-treated samples stored in clay pot, whereas these parameters remained unchanged in samples treated with neem leaves. All these parameters were found to be negatively correlated with infestation at 5% level of signficance.

Keywords: Redgram, Density, Bulk density, Storage, Storage structure, Protectants.

Redgram (*Cajanus cajan*) commonly known as *tur* and *arhar* in major part of India undergoes quantitative changes, when stored. Reduction in weight in foodgrains leads to deterioration in the overall quality of stored grains. The reasons for the loss of weight in foodgrains on storage are drying of grain, activities of insects, birds, rats, consumption of a portion of the grain by the pests during their life cycle and increase in the metabolic activity in the grain (Hall 1970). Loss of weight in pulse grains during storage has been reported by many researchers (Venkat Rao et al. 1960; Jotwani and Sircar 1964; Rajak and Pandey 1965).

Density of the grains is used to assess the degree of damage occurring to the endosperm due to infestation. If the endosperm of the grain is infested, the weight of grain decreases without exerting much effect on the volume of the grain. Subsequently, the ratio of weight to volume (density) decreases. Density of grains was reported to decrease on storage (Pingale et al. 1956; Vimala and Pushpamma 1983). Singh et al (1988) reported that bulk density decreased from 0.916 to 0.829, when greengram was stored under for 4 months, using ash as a protectant.

Since the information on the quantitative changes on weight, density and bulk density of pulses is scanty, the present investigation was designed to study the weight, density and bulk density of redgram stored under different storage conditions.

Freshly harvested redgram (variety 'IPCL-87') was procured from the Department of Agronomy, College of Agriculture, Marathwada Agricultural University, Parbhani in one lot. After cleaning, the lot was divided into 4 equal portions. From one portion of redgram, a sample of 8 kg, without any protectant was stored as a control in clay pot and tin container of 10 kg capacity each. The same amount of redgram from other portions was stored in each storage structure with protectants like dried neem leaves (covering the top surface of grains evenly), cow dung ash (1.0%) and mustard oil (0.5%). The storage was continued up to 4 months. The room temperature during the storage period varied from 25°C to 31°C and relative humidity ranged from 29 to 60%. The moisture content of redgram prior to storage was 10.2%. During the test period, it varied from 8.7 to 13.4%.

An amount of 1 kg was taken from the central portion of each structure at an interval of 2 months for quality evaluation. Weight and density were determined by the method of Pillai et al (1975). Bulk density was measured by the method described by Singh et al (1988).

The effect of storage periods, storage structures and protectants on weight, density and bulk density was determined by analysis of variance of three way classification. Correlation analysis was carried out to find out the relationship between these parameters (weight, density and bulk density) and infestation by the procedure described by Snedecor and Cochran (1956).

Weight, density and bulk density of treated and untreated redgram samples stored for varying periods in different storage structures are presented in Table 1. The weight of 100 seeds of redgram prior to storage was 10.7 g, which was significantly different from that of infested redgram. Two months after storage, the weight varied from 10.1g to 10.7g,

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TABLE 1. WEIGHT, DENSITY, BULK DENSITY AND PROPORTION OF INFESTATION OF TREATED AND UNTREATED REDGRAM STORED FOR VARIOUS PERIODS IN DIFFERENT STORAGE STRUCTURES

Storage structure	Protectants	Storage period							
		2 months				4 months			
		Weight, g/100 seeds	Density, g/cc	Bulk density, g/cc	Infestation, %	Weight, g/100 seeds	Density, g/cc	Bulk density, g/cc	Infestation, %
Clay pot	Control	10.6	1.56	1.25	ND	10.6	1.56	1.25	ND
	Neem leaves	10.6	1.56	1.25	ND	10.6	1.56	1.25	ND
	Cow dung ash	10.1	1.56	1.25	19.2	8.7	1.39	1.11	61.5
	Mustard oil	10.3	1.56	1.25	ND	10.3	1.56	1.25	ND
Tin	Control	10.7	1.56	1.25	8.0	10.5	1.56	1.25	12.3
	Neem leaves	10.6	1.56	1.25	ND	10.6	1.56	1.25	ND
	Cow dung ash	10.3	1.56	1.25	17.5	9.3	1.47	1.18	42.0
	Mustard oil	10.2	1.56	1.25	ND	10.3	1.56	1.25	ND

Each value is the mean of 6 observations. ND : Not detected

while it varied from 8.7g to 10.6g after 4 months of storage. The weight of redgram was the lowest in cow dung ash-treated sample of clay pot. A slight decrease (10.2 g to 10.3g) was noticed in mustard oil-treated sample of both storage structures throughout the storage period, whereas the weight of samples treated with dried neem leaves was 10.6 g in both structures, after 2 and 4 months of storage.

Density and bulk density of redgram prior to storage were 1.67 g/cc and 1.33 g/cc, respectively,

TABLE 2. EFFECT OF STORAGE PERIODS, STORAGE STRUCTURES AND PROTECTANTS ON WEIGHT, DENSITY, BULK DENSITY AND PROPORTION OF INFESTATION

	Weight, g/100 seeds	Density, g/cc	Bulk density, g/cc	Infestation, %
Storage period				
2 months	10.5	1.56	1.25	5.6
4 months	10.1	1.52	1.23	14.5
SE	0.53	0.08	0.06	0.38
CD	NS	NS	NS	1.04
Storage structure				
Clay pot	10.3	1.54	1.24	10.1
Tin	10.4	1.55	1.24	10.0
SE	0.53	0.08	0.06	0.38
CD	NS	NS	NS	NS
Protectants				
Control	10.7	1.56	1.25	5.1
Neem leaves	10.6	1.56	1.25	ND
Cow dung ash	9.6	1.50	1.20	35.1
Mustard oil	10.3	1.56	1.25	ND
SE	0.74	0.11	0.09	0.53
CD	NS	NS	NS	1.47

NS : Not significant, ND : Not detected

which were significantly different from those of infested samples. A significant decrease in density (1.39 g/cc) and bulk density (1.11 g/cc) was noticed only in cow dung ash-treated samples of clay pot after 4 months of storage. A considerable decrease in weight, density and bulk density was observed with advanced period of storage. The cow dung ash-treated samples got infested only after 2 months of storage in both structures. As a result, there was continuous decrease in weight, density and bulk density of samples. The performance of control sample was better than cow dung ash-treated samples, though there was infestation in the control samples stored in tin containers.

The samples treated with mustard oil and neem leaves did not show any infestation even after 4 months of storage. But, the mustard oil-treated samples showed a slight decrease in weight of grains. The weight, density and bulk density of samples treated with dried neem leaves, remained unchanged in both storage structures even after 4 months of storage. Hence, redgram for 4 months either in clay pots or in tin containers can be stored with dried neem leaves, as an effective protectant.

The effect of period of storage, storage structures and protectants on weight, density and bulk density was not significant (Table 2). The infestation of redgram showed a highly significant negative correlation with weight (r= – 0.924), density (r= – 0.836) and bulk density (r= – 0.851) at 5% level.

References

Hall DW (1970) Handling and storage of foodgrains in tropical and sub-tropical areas. Food and Agriculture Organisation of United Nations, Rome. No 90, p 200

Jotwani MG, Sircar P (1964) Studies on the extent of insect damage and germination of seeds. I. Germination of pea seeds damaged by *Callosobruchus maculatus* Fab. Ind J Entomol 26:130-132

Pillai SP, Sharangapani NV, Majumdar SK, Amla BL (1975) Artifacts in the regulatory analysis of commercial samples of foodgrains using uric acid content as a parameter for quality. Int Biodetn Bull 11:4-6

Pingale SV, Kadkol SB, Swaminathan M (1956) Effect of insect infestation on stored *Bengal*gram and greengram. Food Sci 5:211-213

Rajak RL, Pandey ND (1965) A life history study of the pulse beetle. Lab Dev 3:119-122

Singh SP, Kerure AE, Gunjal BB (1988) Effect of treatments on storability of greengram grain. Research Paper, Department of Food Engineering, College of Agric Tech, MAU Parbhani

Snedecor GW, Cochran WG (1956) Statistical Methods. The Iowa State College Press, Ames, Iowa, USA

Venkat Rao S, Nuggehalli RM, Pingale SV, Swaminathan M, Subramanyan V (1960) Effect of insect infestation on stored field bean (*Dolichos lablab*) and blackgram (*Phaseolus mungo*). Food Sci 9:79-80

Vimala V, Pushpamma P (1983) Storage quality of pulses stored in three agroclimatic regions of Andhra Pradesh I. Quantitative changes. Bull Grain Tech 21:54-61

Received 20 September 1995; revised 25 January 1997; accepted 28 January 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 3, 240-241

Occurrence of Keto Fatty Acid in *Hibiscus Ficulneus* Seed Oil

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A keto fatty acid (9-keto-octadec-*cis*-12-enoic acid) in appreciable amounts (280 mg/g) has been isolated from *Hibiscus ficulneus* seed oil. The keto fatty acid was identified by chemical and spectroscopic methods.

Keywords: *Hibiscus ficulneus*, Malvaceae, Seed oil, Keto fatty acid, 9-keto-octadec-*cis*-12-enoic acid.

Hibiscus ficulneus is a branched, prickly annual plant of 6-14 feet in height, found throughout India (Wealth of India 1959). The mucilagenous extract of the green stem is an efficient clarifier for sugarcane juice. The fruits are rich in vitamin C and seeds are aromatic and used for flavouring sweetmeats.

Seed oils containing keto fatty acids are commercially exploited in the paint industry (Swern 1979). *Hibiscus ficulneus* seed oil is a moderate source of keto fatty acid, which shows promise for its exploitation for industrial utilization. Hence, the present investigation was undertaken to identify and quantitate the keto acid from *Hibiscus ficulneus*.

Air-dried seeds were extracted with petroleum ether. The analytical values so obtained were determined according to AOCS (1973) methods and are listed in Table 1. Methyl esters were prepared by refluxing the oil in acidified MeOH (Furniss et al. 1989). Saponification of oil was carried out by stir ring overnight with 0.8M alcoholic KOH. The non-saponifiable matter was removed by extracting with diethyl ether. The mixed fatty acids were partitioned according to the method of Bharucha and Gunstone (1955) between petroleum ether and 80% MeOH. A sample of pure keto acid was obtained by TLC.

Oxidation of the unsaturated acid was carried out in t-butyl alcohol (20 ml). A solution of acid (0.25%) was treated with a solution of $NaIO_4$ (200 mg) in 20 ml of H_2O and $KMnO_4$ (1ml) in the presence of K_2CO_3 (60 mg). The mixture was stirred at room temperature for 24h and the solution then decolorized with $NaHSO_3$, followed by acidification with HCl. The fatty acids were extracted with diethyl ether, the solvent was removed and the acids were treated with 1% H_2SO_4 in MeOH (20 ml). The mixture was refluxed for 1 h and then extracted with diethyl ether. The extracts were dried over Na_2SO_4 and the solvent was removed under reduced pressure. GC analysis showed methyl hexanoate as one of the products and had the same R_t as that of the authentic hexanoate.

Hydrogenation and oxidation of the keto acid were carried out as described. GC analysis of the product showed methyl azeleate as one of the products and had the same R_t as that of an authentic azeleate.

IR spectra were recorded in Hitachi 270-30 spectrophotometer. The NMR spectra were obtained on a Varian T-60 MHz instrument. The chemical shifts (δ) were measured in ppm downfield from internal tetramethyl silane. The mass spectrum was recorded on Joel-JMS-D-300 model instrument. The GLC analysis was carried out in a Perkin-Elmer Model Sigma Unit with a column containing 15% DEGS on Chromosorb W, 354-250 µm (46-60 mesh). The temperatures at injection port, detector port and oven were 240°, 240° and 190°C, respectively. The nitrogen flow and chart speed were 30 ml min^{-1} and 1 cm min^{-1}, respectively.

The oil did not test positive to Halphen (1979) and picric acid TLC (Fioriti and Sims 1968) tests, indicating the absence of cyclopropenoid and epoxy fatty acids, respectively. However, the oil showed positive response to DNPH test (Davis et al. 1969), showing the presence of a keto group. The *Hibiscus*

TABLE 1. ANALYTICAL DATA AND FATTY ACID COMPOSITION OF *HIBISCUS FICULNEUS* SEED OIL

Oil content, %	28.1
Unsaponifiable matter, %	2.3
Saponification value	203.5
Iodine value	51.2
Fatty acids	
Myristic, %	20.2
Stearic, %	21.5
Oleic, %	30.2
9-keto-octadec-*cis*-12-enoic, %	28.1

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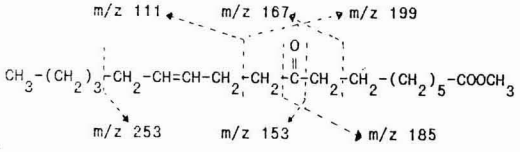
ficulneus seed oil responded to DNPH test (Davis et al. 1969), indicating the presence of a keto group.The IR spectrum of the corresponding methyl ester exhibited characteristic double carbonyl peaks at 1740 cm^{-1} (ester–C(=O)–) and 1705 cm^{-1} (chain–C(=O)–). The IR spectrum also showed bands at 715 and 1620cm^{-1}, for *cis* double bonds. At 970-960cm^{-1} no absorbance for a *trans* double bond could be detected. The ^1H NMR spectrum of the isolated methyl ester of the keto acid exhibited a multiplet at δ 5.38 (2H,–CH=CH–), a second multiplet at δ 2.25 (2H, $-CH_2-CO_2$) and a third multiplet at δ 2.11 (4H, –C(=O)– CH_2 $-CH_2-$C(=O)–), apart from usual proton signals. The acid on hydrogenation (Vogel 1959) with Pd-C furnished 9-keto-octadecanoic acid (m.p.81-82°C). Oxidation [Von Rudloff 1956] of the unsaturated acid with $KMnO_4$–$NaIO_4$ in t butanol, gave hexanoic acid (p-bromophenacyl ester, m.p. 71-72°C) and azelaic acid (m.p. 105-106°C) (p-bromophenacyl ester, m.p. 130-131°C), respectively. There was no depression in the mixed melting point of p-bromophenacyl ester prepared from authentic sample of hexanoic acid and the ester of the hexanoic acid obtained during degradation. The structure of keto acid was further supported by its mass spectrometry. The mass spectrum gave molecular ion peak at m/z 310 [M$^+$] (3.8%), 153 (9%) and 185 (15%); an α-cleavage fragment on either side of the keto group, at 200 (8%) and 168 (14%) (McLafferty cleavage ions on both sides of the keto group). These 4 ions locate the keto group at C-9. Further, an allylic cleavage at m/z 253 (79%) and 111 (14%), was observed. The other important ions were observed at m/z 154 (11%), 110 (41%), 139 (22%), 261 (61%), 290 (43%), 92 (25%), 95 (24%), 51 (40%), 64 (34%), and 71 (base peak). All these observations showed that the original acid is 9-keto-octadec-*cis*-enoic acid. (Scheme 1.)



Scheme – I

Analysis. Carbon 73.35% (required 73.52%), hydrogen 11.05% (required 11.03%), molecular formula $C_{19}H_{34}O_3$. IR: 1740 cm^{-1} ($-CO_2Me$) and 1705 cm^{-1} (chain carbonyl), *cis* double bond absorption at 715 and 1620 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.90t (3H, Me), 1.28 brs (16H, $-CH_2-$chain), 1.95 m (4H,$-CH_2-C=C-CH_2$). 2.11 m (4H,$-CH_2-$C(=O)$-CH_2$), 2.25m (2H, CH_2-CO_2),3.75 s (3H,OMe), 5.38m (2H,–CH=CH). MS m/z 310 [M]$^+$ (Scheme I.) Hydrogenation (Vogel 1959) was carried out using 10% Pd-C in EtOH (4ml) to give 9-keto-octadecanoic acid (m.p. 81-82°C). ^{1}HNMR δ 0.90 (3H, Me), 2.11 (4H, $-CH_2-CO-CH_2$), 2.25 (2H, CH_2-CO_2) and 3.75 (3H, OMe). MS:m/z 312 [M]$^+$. α-cleavage fragments at 155 and 185.

References

AOCS (1973) Official and Tentative Methods of Analysis, Amercian Oil Chemists' Society, 3rd edn, Champaign, IL

Bharucha KE, Gunstone FD (1955) Vegetable oils IV. A new method of determining the component acids of oil containing epoxy and hydroxy acids. J Sci Food Agric 6:373-380

Davis EN, Wallen LL, Goodwin JC, Rohwedder WK, Rhodes AR (1969) Microbial hydration of *Cis*-9-alkenoic acids. Lipids 4:356-362

Fioriti JA, Sims RJ (1968) A spray reagent for the identification of epoxides on thin layer plates. J Chromatogr 32:761-763

Furniss BS, Hannaford AJ, Smith PWG, Tathell AR (1989) Vogel's Textbook of Practical Organic Chemistry, Revised Vth edn, p 699, Longman Group, UK

Halphen G (1979) In: Swern D (ed) Bailey's Industrial Oil and Fat Products Vol.I, p 262. John Wiley and Sons, New York

Swern D (1979) Bailey's Industrial Oil and Fat products, Vol. I p 42, John Wiley and Sons, New York

Vogel AI (1956) A Text Book of Practical Organic Chemistry 3rd ed,n pp 578, 866 and 950, Longmans Green and Co. Ltd., London

Von Rudloff E (1956) Oxidation of lipids in media containing organic solvents. Can J Chem 34:1413-1418

Wealth of India, (1959) Raw Materials Vol. V, Council of Scientific and Industrial Research, New Delhi, pp 88-89

Received 14 June 1996; revised 25 January 1997; accepted 28 January 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 3, 242-244

Effect of Storage and Ripening on Peroxidase Enzyme, Some Nutritional and Antinutritional Factors Present in the Pulp and Seeds of Pumpkin (*Cucurbita maxima*)

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The biochemical effects of storage and ripening on pumpkin pulp and seeds were studied with respect to peroxidase enzyme, various nutritional and antinutritional factors. Storage and ripening affected the peroxidase enzyme activity, free amino acids, proteins, total sugars and phenols.

Keywords: Peroxidase enzyme, Pumpkin pulp, Pumpkin seeds, Storage, Ripening, Nutritional factors, Antinutritional factors.

Peroxidases (donor: hydrogen peroxide: oxidoreductase E.C. 1.11.1.7) are lytic enzymes, which are widely distributed among the plant kingdom. It acts as a detoxicant and its role is much more related with the storage of fruits and vegetables (Haplin et al. 1989). It has been studied in different fruits and vegetables like grapes (Kumar et al. 1987) and Kiwi fruit (Guadalupe 1989). Pumpkin is a popular vegetable from cucurbitaceae family in India. Its pulp is eaten as unripe and ripe. Though the seeds are nutritionally important and have medicinal value, they are thrown away (Samba Murthy 1989). Therefore, attempts were made to study peroxidase enzyme activity, optimum conditions of enzyme, activators and inhibitors of enzyme, nutritional and antinutritional factors in pulp and seeds of pumpkin (unripe and ripe).

Pumpkin pulp and seeds were bought fresh from the local market. After cleaning, the enzyme was extracted and estimated by the modified method of Nagar et al (1993). Proteins were assayed by the method of Lowry et al (1961), as modified by Khanna et al (1969). Phenols were estimated by the modified method of Goldstein and Swain (1963). Total sugars were determined by the method of Dubois et al (1956). The free amino acids were estimated by the method introduced by Plummer (1986). All readings were taken in triplicate and each analysis was done in three replicates. Katf values were calculated in terms of fresh weight and protein by the modified method of Nagar et al (1993). Katf value can be described as velocity constant K at 'O' time/ml of enzyme extract as well as mg of protein/ml of sample, respectively.

The optimum pH and temperature of peroxidase enzyme were 7 and 35°C, respectively both in pumpkin pulp and seeds. Km and substrate concentration of enzyme were 0.98 gm/l and 0.1N in pulp, while in seeds, they were 1.4 gm/l and 0.08 N. Katf in terms of fresh weight was 0.02 and 0.34 in pulp and seeds, while in terms of protein it was 0.83 and 0.82, respectively.

Effect of some organic and inorganic compounds (0.5% concentration) as % inhibition/% activation of peroxidase enzyme was studied. Potassium chlorid NaOH, and Na_2SO_4 were found activators of enzyme both in seeds and pulp. Calcium Chloride and oxalic acid were found activators in pulp, while these were inhibitors of enzyme in seeds. Glycine, KOH, $MgCl_2$, $MgSO_4$ and ascorbic acid were found as inhibitors of enzyme both in pulp and seeds. Maximum % inhibition was found by $MgCl_2$ and ascorbic acid on enzyme of seeds and pulp, respectively. Ascorbic acid showed

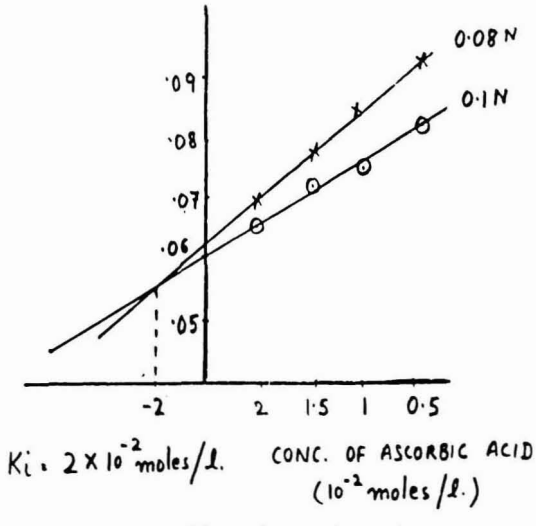

Fig. 1. Competitive inbibition by ascorbic acid

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TABLE 1. PEROXIDASE ENZYME ACTIVITY, DIFFERENT NUTRITIONAL AND ANTINUTRITIONAL FACTORS IN PULP AND SEEDS (UNRIPE AND RIPE) AT DIFFERENT STORAGE TIME.

Days	Enzyme activity		Amino acids, µM/g		Protein, mg/g		Total sugars, µg/g		Phenols, mg/g	
	UR	R	UR	R	UR	R	UR	R	UR	R
					Pulp					
1.	7.5 (62.5)	8.9 (39.03)	3.6	8.9	0.48	0..91	21.2	25	60.0	0.15
2.	9.1 (86.6)	11.4 (54.28)	-	-	0.42	0.84	-	-	72.01	0.07
3.	10.03 (154.3)	11.6 (91.33)	-	-	0.26	0.50	-	-	0.01	0.03
4.	10.34 (240.4)	11.91 (109.26)	8.8	23.3	0.17	0.43	39.6	70.6	0.01	0.01
5.	11.1 (308.3)	12.23 (174.71)	-	-	0.14	0.28	-	-	0.00	0.01
6.	12.22 (555.4)	15.68 (412.6)	-	-	0.09	0.15	-	-	0.00	0.00
7.	-	-	22	55	-	-	67.4	121	-	-
					Seeds					
1.	2.19 (2.19)	2.19 (1.25)	110	450	5.00	8.75	5	47.5	1.50	82.0
3.	5.64 (12.5)	5.64 (3.4)	-	-	-	-	-	-	-	-
4.	-	-	-	-	2.25	8.15	-	-	1.42	65.0
6.	8.05 (38.3)	13.17 (36.5)	-	-	1.80	1.80	-	-	1.22	33.0

Readings were not taken on that day

Specific activity values of enzyme are given in parenthesis

UR Unripe

R Ripe

competitive inhibition with inhibitor constant Ki as $2x10^{-2}$ moles/litre (Fig.1). Different researchers have studied different inhibitors (Agarwala et al. 1979) and activators (Chatterjee et al. 1989) of peroxidase enzyme.

Calcium chloride and oxalic acid were found inhibitiors in seeds and activators in pulp. Km, substrate concentration and Katf values were also different in pulp and seeds as described previously. Thus, localisation of the enzyme can affect the character of the enzyme. Different types of peroxidase enzymes have also been reported from the same tissue (Grison and Pilet 1979).

Enzyme activity and the specific activity in unripe and ripe seeds were found to be in an increasing order with storage. In pulp, only the specific activity was noted to be in an increasing order. (Table 1). An increase in peroxidase enzyme with storage time was also found in cucumber (Miller et al. 1989). Due to ripening, in pulp and seeds, the specific activity decreased, while no significant increase was seen in enzyme activity. Increase in peroxidase enzyme, due to ripening was reportedin tomato (Rottan and Nicholas 1989).

On storage, the protein and phenol decreased in pulp and seeds, while total sugars increased in the pulp (unripe and ripe). Fuke and Matsuuoka (1984) also reported decrease in phenols, which remained constant during ripening, but an increase was reported in grapes with maturation (Kumar et al. 1987).

With ripening the nutritional factors free amino acids and total sugars) increased in pulp and seeds, while the antinutritional factors (phenols) decreased in pulp, but increased in seeds.

References

Agarwala SC, Sharma PN, Nautiyal BB, Sharma CP (1978) Effect of zinc supply on the growth and activity of certain enzymes in barley growth in sand culture. Indian J Plant Physiol 21:181-184

Chatterjee C, Sinha P, Nautiyal N, Kurana N, Agarwala SC (1989) Physiological response of cauliflower in relation to Mn-Boron interaction. J Hortic Sci 64:591-596

Dubois M, Gilles KA, Hamilton JK, Rabes PA, Smith F (1956) Colorimetric method for the determination of sugars and related substances. Anal Chem 28:350-354

Fuke Y, Matsuuoka H (1984) Changes in content of pectic substances, ascorbic acid and polyphenols and activity of pectinesterase in kiwi fruit during growth and ripening after harvest. J Jap Soc Food Sci Tech 312(1):31-37

Goldstein JN, Swain T (1963) Changes in tannins in ripening fruits. Phyto Chem 2:371-383

Grison R, Pilet PE (1979) Critical analysis of corn root peroxidase plant. Sci Lett 13:213-218

Guadalupe P (1989) Peroxidase of kiwi fruit, J Food Sci 54(3)760-762

Haplin B, Pressey R, Jen J, Mondy N (1989) Purification and characterisation of POD isoenzyme from green peas (*Pisum sativum*). J Food Sci 54(3)644-649

Khanna SK, Matto RL, Vishwanathan PN, Tiwari CP, Sanwal GG (1969) Colorimetric determination of protein and orthophosphate in plant tissue rich in phenolics, Ind J Biophys 21:949-950

Kumar S, Singh A, Singh AB (1987) Oxidative enzymes during maturation and ripening of grapes. Sci Culture 53 (12): 381-383

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1961) Protein measurement with the Folin phenol reagent. J Biochem 193:265-275

Miller A, Raymond K, Thomas J (1989) Mechanical stress stimulates peroxidase activity in cucumber fruit. Horticult Sci 24(4):650-652

Nagar R, Bhargava M, Nagar PN (1993) Relation between peroxidase activity, protein and phenolics at different levels of germinated legumes. J Ind Bot Soc 72:1-3

Plummer DT (1982) An Introduction to Practical Biochemistry. 2nd edn. Tata McGraw Hill Publ Co. Ltd. New Delhi

Rottan C, Nicholas J (1989) Changes in acidic and basic proxidase activities during tomato fruit ripening. Horticult Sci 24(2):340-342

Samba Murty AVSS, Subramaniyam NS (1989) A Text Book of Economic Botany. Wiley Eastern Ltd., New Delhi, pp 679-680

Received 5 January 1996; revised 25 January 1997; accepted 28 January 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 3, 245-247

Studies on Packaging and Storage of Cereal-Pulse Based Sweetbars

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Moisture sorption studies carried out at relative humidities ranging from 11 to 92% on 3 different ready-to-eat sweetbars (45x45x9.5 mm) based on pre-processed wheat/pulse flours/*soji* (*semolina*) indicated that equilibrium moisture content (EMC) of 7.0 to 7.7% at 65% RH was critical, beyond which sogginess and mould growth were observed. Sweetbars packaged in pouches of polypropylene (PP) (50 micron) and metallised polyester/polyethylene (Met.PET/PE) had shelf-life upto 150 days at ambient (65% RH and 27°C) condition, while at accelerated (90% RH and 38°C) condition, the products kept well for 90 days in PP pouches and 150 days in Met.PET/PE pouches.

Keywords: Packaging materials, Flexible films, Sweetbars, Shelf-life, Moisture sorption.

In recent years, some of the popular Indian traditional sweetbars (*burfi*-like) or ball-shaped *laddus*, based on cereal/pulse flours are being increasingly exported to the Middle-east countries. Generally, these fat-rich sweetbars have poor shelf-life of only a few days under ambient weather conditions due to discoloration, textural changes, development of rancidity and stale taste and fat bloom. A few studies have been carried out on the preparation and packaging of such products like *sohan papri* (Venkatesh et al. 1983), *sohan halwa* (Venkatesh et al. 1984), *khoa* (Abhoy Kumar et al. 1975) and milk *burfi* (Ramanna et al. 1983).

The present study discusses the moisture sorption characteristics and packaging aspects of 3 different ready-to-eat sweetbars, based mainly on pre-processed wheat *atta* (A), wheat *soji (S)* and *Bengal*gram flour (B) with other ingredients like sugar, milk powder, puffed *Bengal*gram flour, vanaspati, cardamom etc., and prepared (unpublished data) in square shape (45 x 45 x 9.5 mm), using a tablet making machine (UNIMEK, Mumbai, India). Considering the physico-chemical attributes of the products, economy and availability of the packaging materials as well as the product sensitivity to the varying climatic conditions in different regions of the country, 50 µm (200 gauge) unoriented polypropylene (PP) and 12 µm (50 gauge) metallised polyester/37µm (150 gauge) polyethylene laminate (Met. PET/PE) were used for assessing their suitability for packaging and storage studies.

Chemical analysis: Peroxide value (PV, as meq of O_2/kg of fat) and free fatty acids (FFA, as % oleic acid) of the experimental samples were determined in duplicate according to AACC (1976) procedures.

Moisture sorption studies: The relative humidity-moisture relationships of the three sweetbars were studied at 27°C by exposing weighed quantities of the samples in petri dishes to different relative humidities (RH), ranging from 11 to 92%, built up in different desiccators by using appropriate saturated salt solutions (Rockland 1960). The samples were weighed periodically, till they attained constant weight or showed signs of fungal attack, whichever was earlier. After equilibration, the samples were assessed for changes in physical parameters like texture, odour, colour etc. The equilibrium moisture content (EMC) of the sweetbars was calculated on the basis of changes in the moisture contents at different RH conditions and their respective initial moisture contents.

Packaging and storage studies: The water vapour transmission rates (WVTR) of the two packaging materials used were determined according to the IS:1060 (1960) method. Two sweetbars, each weighing about 25g, were placed in 60 x 60, mm pouches, which were then heat, sealed, weighed individually and exposed to (1) overall average Indian weather conditions of 65±2% RH and 27±1° C and (2) accelerated storage conditions of 90±2% RH and 38±1°C.

During the storage under the above conditions, the individual packs of sweetbars were weighed periodically and the contents were analysed for PV and FFA and evaluated for overall acceptability, based on scores for colour (15), breakability (15), aroma (20) and eating quality (50; i.e., mouth feel: 20 and taste: 30) by a trained panel of 6 judges.

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TABLE 1. MOISTURE CONTENT-RELATIVE HUMIDITY RELATIONSHIPS OF SWEETBARS

RH,%	Equilibrium moisture content, %			Quality characteristics
	Atta-based (A)	*Bengal*gram flour based (E)	*Soji*-based (S)	
11	1.4	2.7	2.9	Crisp, acceptable
22	1.7	3.3	3.7	- do -
33	4.7	3.9	4.5	- do -
44	5.5	4.7	5.4	Slightly less crisp, acceptable
56	7.1	6.1	6.5	- do -
65	7.7	7.0	7.2	Just acceptable
75	10.9	10.5	9.1	Soft, soggy, not acceptable
86	11.8	14.3	10.4	Soft, soggy, mould growth not acceptable
92	21.6	20.8	17.3	- do -
Initial (moisture)	4.1	3.6	4.2	Crisp, acceptable

On the basis of overall scores, the samples were graded as excellent (86-100), good (71-85), satisfactory (56-70), fair (41-55) and poor (less than 40).

The results presented in Table 1 show that the three sweetbars based on *atta* (A), *Bengal*gram flour (B) and *soji* (S) with corresponding initial moisture contents of 4.1, 3.6 and 4.2% equilibrated to an RH of about 30%. The moisture sorption isotherm of the products were of sigmoid type similar to that of maize and pulse-based food products and exhibited a considerable rise above 65% RH (Kumar and Anandaswamy 1979). The observations on the quality of these products after equilibration are indicated in Table 1. When stored in the RH range of 11 to 65%, the sweetbars were crisp and acceptable. There was a slight loss in crispness in the case of samples stored at 44 to 56% RH, but were still acceptable. At 65% RH, the products A, B and S with moisture contents of 7.7, 7.0 and 7.2%, respectively were just acceptable, as indicated by development of softness. Hence, a

TABLE 2. CHANGES IN MOISTURE, PV[a], FFA[b] AND OVERALL ACCEPTABILITY (OA)[c] OF SWEETABARS PACKED IN FLEXIBLE POUCHES STORED AT AMBIENT AND ACCELBRATED CONDITIONS

Packaging material	Storage period, days	*Atta*-based				*Bengal*gram flour-based				*Soji*-based			
		Moisture content,%	PV	FFA	OA	Moisture content,%	PV	FFA	OA	Moisture content,%	PV	FFA	OA
Initial	0	3.86	8.4	0.82	Good	3.46	7.8	0.98	Good	4.00	7.4	0.40	Good
Ambient condition													
	30	4.09	8.7	0.82	Good	3.65	8.5	0.99	Good	4.20	8.0	0.55	Good
	60	4.28	9.2	0.96	Good	3.82	9.3	1.00	Good	4.39	8.9	0.58	Good
Polypropylene	90	4.54	10.0	1.00	Good	4.07	10.1	1.00	Good	4.68	9.8	0.61	Good
	120	4.75	10.8	1.01	Good	4.25	10.9	1.06	Good	4.84	10.4	0.64	Good
	150	5.00	11.6	1.10	Good	4.42	11.5	1.10	Good	5.02	11.1	0.84	Good
	30	3.95	8.5	0.82	Good	3.53	8.1	0.99	Good	4.08	7.9	0.44	Good
	60	3.99	8.8	0.84	Good	3.57	8.6	0.99	Good	4.14	8.4	0.50	Good
Met.PET/PE	90	4.06	9.4	0.90	Good	3.64	9.2	1.00	Good	4.19	9.0	0.52	Good
	120	4.11	10.0	0.93	Good	3.69	9.8	1.02	Good	4.25	9.5	0.56	Good
	150	4.18	10.5	0.98	Good	3.74	10.4	1.04	Good	4.31	10.2	0.76	Good
Accelerated condition													
	30	4.47	9.1	0.87	Good	4.00	8.9	0.99	Good	4.58	9.0	0.55	Good
	60	5.10	9.9	1.01	Good	4.58	10.0	1.04	Good	5.16	9.8	0.61	Good
Polypropylene	90	5.72	11.0	1.07	•	5.10	11.2	1.10	•	5.74	10.7	0.93	Good
	120	6.18	12.4	1.10	Poor	5.64	11.9	1.13	Poor	6.26	11.3	0.95	•
	150	6.98	13.3	1.23	Poor	6.13	12.8	1.17	Poor	6.78	12.2	1.07	Poor
	30	4.18	8.8	0.85	Good	3.71	8.5	0.84	Good	4.28	8.6	0.44	Good
	60	4.54	9.6	0.92	Good	3.97	9.1	1.00	Good	4.56	8.9	0.60	Good
Met.PET/PE	90	5.06	10.2	0.93	Good	4.48	9.9	1.01	Good	5.24	9.6	0.66	Good
	120	5.57	10.9	0.96	Good	4.94	10.7	1.03	•	5.63	10.4	0.97	•
	150	6.02	12.0	1.14	Good	5.42	11.9	1.22	•	6.02	11.8	1.03	•

[a]Peroxide value expressed as meq. of O_2/kg of fat, [b]Free fatty acids expressed as % of oleic acid, [c]Based on parameters for colour, breakability, flavour and eating quality

• Satisfactory.

moisture content in the range of 7.0 to 7.7%, which equilibrates to 65% RH was the critical moisture level. The products had shown satisfactory moisture tolerance with a permissible moisture uptake of 3.0 to 3.7% for an acceptable quality. When equilibrated to the RH range of 75 to 92%, the products were relatively soft, soggy and there was mould growth also.

Table 2 shows the changes in moisture content, PV, FFA and overall acceptability of the products, packed in PP and Met. PET/PE laminates under ambient and accelerated storage conditions for the study period of 150 days. Under ambient condition, the moisture pickup was higher in the case of products in PP pouches as compared to those in Met. PET/PE pouches. The WVTR values of the packaging materials were 7.8 and 1.5 g/m²/day at 38°C and 90% RH, respectively. Thus, Met.PET/PE provides better protection against moisture pickup. The peroxide and FFA values varied only narrowly and the overall acceptability was good for all the products in both the pouches at the end of 150 days (Table 2). In the case of the products stored at the accelerated condition, the moisture pickup was markedly higher than those stored at ambient condition, but the moisture content was within the permissible limits of critical moisture level. There was a slight increase in PV and FFA of the products packaged in PP pouches compared to those in Met.PET/PE pouches (Table 2). The overall acceptability of *soji*-based sweetbars was good, while that based on *atta* and *Bengal*gram flour was satisfactory at the end of 90 days storage in PP pouches. In Met.PET/PE pouches, at the end of 150 days, the overall acceptability of *atta*-based product was good and that based on *soji* or *Bengal*gram flour was satisfactory.

The present study has shown that the sweetbars could be stored well for 150 days at ambient condition in both PP and Met.PET/PE pouches, while at accelerated condition, the products could be stored well for 90 days in PP pouches and for 150 days in Met.PET/PE pouches.

The authors thank Mr. K.R. Kumar, Scientist, Food Packaging Department, CFTRI, for help in the preparation of the manuscript, Dr. J.V. Prabhakar, Head and Dr. S. Yella Reddy, Scientist, Department of Lipid Science and Traditional Foods for interest in the work.

References

AACC (1976) Approved Methods, 9th edn. American Association of Cereal Chemists, St. Paul, Minn

Abhoy Kumar, Rajorhia GS, Srinivasan MR (1975) Effect of modern packaging materials on the keeping quality of *khoa*. J Food Sci Tech 12:172-175

IS:1060 (1960) Methods of Sampling and Test for Papers and Allied Products, Part II, Bureau of Indian Standards, New Delhi

Kumar KR, Anandaswamy B (1979) Shelf-life studies on a flour blend based on maize and pulses. J Food Sci Technol 16:118-119

Ramanna BR, Bhat KK, Mahadevaiah B, Dwarakanath CT, Dhanaraj S, Potty VH, Sen DP (1983) Investigations on large scale preparation and processing of milk *burfi*. J Food Sci Technol 20:67-71

Rockland LB (1960) Saturated salt solutions for static control of relative humidities. Anal Chem 32:1375-1376

Venkatesh KVL, Mahadevaiah B, Dhanaraj S, Mahadevaiah M, Ananthakrishna SM, Anandaswamy B, Govindarajan VS, Sen DP (1983) Studies on packaging and storage of *sohan papri*. J Food Sci Technol 20:209-213

Venkatesh KVL, Dhanaraj S, Mahadevaiah B, Ananthakrishna SM, Mahadevaiah M, Anandaswamy B, Govindarajan VS, Sen DP (1984) Effect of packaging on quality of *sohan halwa* during storage. J Food Sci Technol 21:167-171

Received 19 March 1996; revised 29 January 1997; accepted 30 January 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 3, 248-250

Isolation of *Listeria monocytogenes* from Milk

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A total of 121 milk samples comprising 50 individual cow milk of an organised dairy farm, 16 individual cow milk of a private dairy farm, 35 pasteurized bulk tank milk and 20 non-pasteurized bulk tank milk were subjected to detailed bacteriological examination for the isolation of *Listeria monocytogenes*. Out of 16 *Listeria* isolates recovered, 7 were of *L. monocytogenes* (organised dairy farm-2, private farm-4 and non-pasteurized bulk tank milk-1) and remaining 9 isolates belonged to other *Listeria* spp (organised dairy farm-3, private dairy farm-4, non-pasteurized bulk tank milk-2). Pathogenicity testing of *L. monocytogenes* isolates revealed 6 isolates to be pathogenic. The antibiogram studies showed that *L. monocytogenes* were sensitive to doxcycline, ampicillin, ciprofloxacin and penicillin. The presence of pathogenic *L. monocytogenes* in milk may cause serious health problem to human.

Keywords: *L. monocytogenes*, Prevalence, Milk, Antibiogram, Pathogenicity.

Listeria monocytogenes is a dangerous pathogenic microorganism to man and animals. The organism can grow and multiply at refrigeration temperature and thus can be a potential microbiological risk from various refrigerated foods. Its wide distribution in nature makes the presence of bacteria in foods of animal origin almost unavoidable (Johnson et al. 1990). In recent years, outbreaks of serious foodborne listeriosis, resulting in many deaths have been associated with the consumption of milk and milk products (Fleming et al. 1985, Linnan et al. 1988). This has led to a renewed scientific interest on *L. monocytogenes*.

The emergence of foodborne listeriosis as a potent public health problem has prompted the WHO to suggest that the presence of *L. monocytogenes* in various foods should be investigated on world wide basis and the contamination of foods with *L. monocytogenes* should be controlled (WHO 1988). In India, although *Listeria monocytogenes* has been isolated from several listeriosis cases of animals (Dhanda et al. 1959; Phadke et al. 1979; Vishwanathan and Uppal 1981; Kulshrestha et al. 1975; Srivastava et al. 1985), the exact status of prevalence of the organism in foods of animal origin is still unclear. Hence, the present work was undertaken to assess the prevalence of *L. monocytogenes* in milk.

Sample collection : A total of 121 milk samples viz., individual cow milk of organised farm-50, individual cow milk of private dairy farm-16, pasteurized bulk tank milk-35, non-pasteurized bulk tank milk-20 were collected aseptically from various sources in and around Bareilly. The samples were immediately transported to laboratory on ice in thermos flask and were processed within 1-2 h of procurement.

Isolation of Listeria monocytogenes : The USDA procedure (Mcclain and Lee 1988) with modification was adopted for the isolation and identification of *L. monocytogenes*. The enrichment of milk samples was done in University of Vermont broth (UVM) I and II (Donnelly and Baigent 1986). However, for selective plating, Dominiguez Rodriguez Agar (DRA) (Dominiguez Rodriguez et al. 1984) was used.

Ten ml milk sample was mixed with 90 ml UVM-I broth and was incubated at 30°C for 48h. From this, 0.1 ml inoculum was transfered to UVM-II broth and after 48h of incubation of UVM-II broth at 30°C, a loopful inoculum was streaked on DRA plates. The plates were incubated at 37°C for upto 48h. Typical esculin positive colonies of *Listeria monocytogenes* i.e., showing black zone of esculin hydrolysis around colonies were first screened for morphology, staining, motility and catalase activity and were further subjected for biochemical characterization (Smith et al. 1990). Biochemical tests carried out were methyl red test, voges prausker test, fermentation of mannitol, xylose, rhamnose, methyl-D-α mannoside, nitrate test, haemolysis on the sheep blood agar and CAMP test with *staphylococcus aureus*. Confirmed isolates were maintained on brain heart infusion (BHI) agar slant.

Pathogenicity testing: Confirmed isolates of *L. monocytogenes* were subjected to pathogenicity testing, using mice pathogenicity test and Anton test (Killinger 1974). The mice pathogenicity test was carried out on mice, weighing 18-20g. The i/p injection of 0.2ml of 24h BHI broth culture resulted in death within 48-72h. The organisms could be recovered from heart blood and spleen.

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TABLE 1. ISOLATION OF *LISTERIA* ORGANISM FROM MILK SAMPLES

Source of sample	No.of samples examined	No. of positive samples	
		L. monocyto-genes	*Other Listeria* spp
Organised dairy farm	50	2 (4)	3 (6)
Private dairy farm	16	4 (25)	4 (25)
Pasteurized bulk tank milk	35	Nil	Nil
Non-pasteurized bulk tank milk	20	1 (5)	2 (10)
Total	121	7 (5.78)	9 (7.44)

* Figures in parenthesis indicate % of positive samples

TABLE 2. ANTIBIOTIC SENSITIVITY PATTERN OF *L. MONOCYTOGENES* ISOLATES

Antibiotics	Conc/disc	No. of isolates[a] sensitive
Doxycyline	30 mcg	7
Neomycin	30 mcg	Nil
Amoxycillin	10 mcg	4
Ampicillin	10 mcg	7
Ciprofloxacin	5 mcg	7
Gentamicin	10 mcg	4
Nitrofurantoin	300 mcg	3
Trimethoprim	5 mcg	5
Penicillin G	10 units	6
Spiramycin	30 mcg	2
Cephalexin	30 mcg	Nil

[a] No. of isolates tested are seven.

The Anton's test showed purulent conjunctivitis within 48h after instillation of 1 drop of 18h old culture of *L. monocytogenes*. Pathogenicity testing revealed 6 of the 7 *L. monocytogenes* isolates to be pathogenic.

Antibiogram: Antibiotic sensitivity pattern of *L. monocytogenes* isolates was studied by disc diffusion method (Matsen and Barry 1974), using various antibiotic discs.

Results of the study indicated that out of the 121 milk samples screened, 7.44% samples were positive for *Listeria* spp, whereas 5.78% samples had *L. monocytogenes* (Table 1). Highest incidence of *L. monocytogenes* was found in milk samples collected from the private dairy farm (25%), followed by 5% in the non-pasteurized bulk tank milk and 4% in milk samples of the organised dairy farm. All the pasteurized bulk tank milk samples were negative for *Listeria* organisms. Results of the study are in agreement with earlier reports on contamination of bulk tank milk samples. (Rohrbach et al. 1992) and raw milk samples (Lovett et al. 987) with *L. monocytogenes*. Greenwood et al. (1991) have reported 1% incidence of *L. monocytogenes* in pasteurized cow milk, whereas in the present study, none of the pasteurized milk samples contained listeriae. High prevalence of *L. monocytogenes* in milk from the private dairy farm reported in this study reflects the improper milking/handling, unhygienic conditions and improper husbandry practices in the farm. The husbandry practice such as ingestion of contaminated feed, improper health management of animals have been reported to be responsibe for the higher incidence of *Listeria monocytogenes* in milk (Skovgaard 1989).

The results of the antibiotic sensitivity pattern as shown in Table 2, indicate that all the *L. monocytogenes* isolates were sensitive to doxycyline, ampicillin and ciprofloxacin. The antibiotics like penicillin-G, and trimethoprim were also effective against majority of the isolates of the present study. This susceptibility pattern will be helpful in formulating strategy for treatment of listeriosis.

Incrimination of food products, especially dairy products in the outbreaks of listeriosis, has emphasized the need for systematic control of *L. monocytogenes* in the raw milk. The occurrence of pathogenic *L. monocytogenes* in the milk samples screened in the present study underlines the fact that attention must be given to the hygiene measures in animal rearing, feeding, milking procedures, processing, storage and distribution of milk (Anon 1989). Since pasteurization eliminates the organisms (Bradshaw et al 1991), proper pasteurization and careful handling is recommended to avoid risk of milk borne infection.

The authors acknowledge the help provided by Head, Veterinary Public Health, IVRI to carry out this study and Mr. K.K. Bhat for technical help and also for typing the manuscript.

References

Anon (1989) Conclusions of the panel meeting held at the Tenth International Congress on Listeriosis. Int J Food Microbiol 8:181-182

Bradshaw JG, Peeles JT, Twedt RM (1991) Thermal resistance of *Listeria* spp in milk. J Food Prot 54:12-14

Dhanda MR, Lall JM, Seth R, Chandrasekariah P (1959) A case of Listeric abortion in an ewe with a small scale survey of the incidence of agglutinins to *Listeria* in the serum of sheep. Indian Vet J 36:113-124

Dominguez-Rodgriguez L., Suarez-Fernandez G, Fernandez-Garayzabal JF, Rodriguezferri EF (1984) New methodology for the isolation of *Listeria* microorganisms from heavily contaminated environments. Appl Environ Microbiol 47:1188-1190

Donnelly CW, Baigent GK (1986) Method for flow cytometric detection of *Listeria monocytogenes* in milk. Appl Environ Microbiol 52:689-695

Fleming DW, Cochi SL, MacDonald KL, Brondun J, Hayes PS, Plikaytis BD, Holmes MB, Audurier A, Broome CV, Reingold AL (1985) Pasteurized milk as a vehicle of infection in an outbreak of Listeriosis. N Engl J Med 321:404-407

Greenwood MH, Roberts D, Burden P (1991) The occurrence of *Listeria* species in milk and dairy products. A National survey in England and Wales. Int J Food Microbiol 12:192-206

Johnson LJ, Dole MP, Cassens RG (1990) *Listeria monocytogenes* and other *Listeria* spp in meat and meat products. A review. J Food Prot 53:81-91

Killinger AH (1974) *Listeria monocytogenes* in: Lennette EH, Spaulding EH, Truant JP (eds) Manual of Clinical Microbiology. 2nd edn, American Society for Microbiology, Washington, DC pp135-139

Kulshrestha SB, Paliwal OP, Lal Krishnan (1975) A note on Listeric encephalitis in sheep. Indian J Anim Sci 45:804-806

Linnan MJ, Macol L, Lou XD, Goulet VG, May S, Salminen C, Hird DW, Hayes P, Yonekura ML, Weaver R, Audurier A, Plikaytis BD, Finnin SL, Klek A. Proome CV (1988) Epidemic listeriosis associated with Mexican-style cheese. N Engl J Med 329:823-828

Lovett J, Francis DW, Hunt JM (1987) *Listeria monocytogenes* in raw milk: Detection incidence and pathogenicity. J Food Prot 50:168-192

Matsen JM, Barry AL (1974) Susceptibility testing: Diffusion test procedures. In: Lennette EH, Spauding EH, Truant JP (eds) Manual of Clinical Micriobiology, 2nd edn, American Society for Microbiology, Washington DC pp418-427

Mclain D, Lee WH (1988) Development of USDA-FSIS method for isolation *Listeria monocytogenes* from raw meat and poultry. J Assoc Off Anal Chem 71:660-664

Phadke SP, Bhagwat SV, Kapshikar R, Ghevari SD (1979) Listeriosis in sheep and goats in Maharashtra. Indian Vet J 5:634-637

Rohrbach BW, Draughon FP, Davidson M, Oliver SP (1982) Prevalence of *Listeria monocytogenes*, Campylobacter jejuni, Risk factors and risk of human exposure. J Food Prot 55(2):93-97

Skovgaard N (1989) HACCP approach to the concept at farm level. In: Proceedings of World Association of Veterinary Food Hygiene (WAVFH) Xth (Jubilee) International Symposium: Healthy Animals-Safe Food-Healthy Man. 2-7 July, 1989

Smith GR, Mcgauchlin J, Taylor AG (1990) Erysipelothrix and Listeria In: Parker TM, Duerden BI (eds), Topley and Wilsons Principles of Bacteriology, Virology, and Immunity, 8th Edn. Vol. 2. Systematic Bacteriology. Edward Arnold, Division of Hodder and Stoughton, London

Srivastava NC, Gupta SK, Uppal PK (1985) *Listeria monocytogenes* infection in infertile cows. Indian Vet J 62:545-547

Vishwanathan KR, Uppal PK (1981) Isolation of *Listeria* from sheep. J Remount Veterinary Corps. 20:127-130

WHO (1988) World Health Organization Working Group. Foodborne Listeriosis. WHO. Bull 66:421-428

Received 10 July 1995; revised 30 January 1997; accepted 31 January 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 3, 251-254

Effect of Incorporation of Sorghum Flour to Wheat flour on Chemical, Rheological and Bread Characteristics

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Sorghum flours (quality grades-25 and 15% polishing) were incroporated into wheat flour at 5,10,15 and 20% levels. The water absorption of flour blends and dough strength decreased with the increase in the level of sorghum flour. The bread volume decreased with increasing level of sorghum substitution. The crumb colour changed from creamish white to dull brown and a gradual hardening of crumb texture was observed as the addition of sorghum increased. Replacement of wheat flour upto 15 and 10% with 75 and 85% extraction rate sorghum flours, respectively produced acceptable breads.

Keywords: Refined sorghum flour, Extraction rate, Dough characteristics, Composite flour bread.

Sorghum (*Sorghum vulgare. L*) and other millet grains hold an important place in the foodgrain economy of India. The world sorghum production is 70,448 metric tonnes and India produces about 13,000 metric tonnes per year, accounting for 18.5% of world sorghum production (FAO Year Book 1992). In many Asian and African countries, sorghum is used for human consumption and is utilised in porridges, unleavened bread and snacks.

Bread is traditionally made from wheat flour. Other cereal flours like rye, barley, sorghum and maize have been used either alone or in combination with wheat flour for breadmaking in various parts of the world (Samuel 1972). Several studies have indicated the possibility of incorporating sorghum in wheat flour at various levels. Such composite flours can be used for producing bread, biscuits and other snacks (Badi et al. 1976, Hart et al. 1970; Hulse et al. 1980). Haridas Rao and Shurpalekar (1976) stated that a biscuit preparation based on 80:20 blend of *maida* and sorghum flour compared with biscuits based only on *maida*. Sumner and Nielsen (1976) produced acceptable Nigerian bread, using an 80/20 wheat/sorghum composite flour blend. Use of sorghum and millet in cookies has also been reported (Badi et al. 1976). Generallly, addition of more than small amounts of sorghum or pearl millet flour decreases loaf volume. However, the flavour of bread containing pearl millet is generally considered excellent (Badi et al. 1976; Casey and Lorenz 1977). Chavan and Kadam (1993) reported in their article that Bankar et al used a white sorghum flour to the extent of 30% for blending with wheat flour to prepare breads and sweet buns. By making more extensive use of cereals, other than wheat, like sorghum, ragi, pearl millet etc., which are grown widely in the country, diversified food products could be prepared.

The objective of this study was to see the effect of incorporation of refined sorghum flour on chemical, rheological and baking characteristics of wheat flour.

Commercial wheat flour (*maida*) and sorghum (white variety) were obtained from the local market. Sorghum was conditioned using 4% water, in a hand-operated mixer for 5 min and allowed to rest for 10 min. The conditioned sorghum was pearled in a rice huller to 25 and 15% degrees of polish. The pearled sorghum was ground in a disc mill to pass through 6XX (Venkateswara Rao et al. 1985). Wheat and sorghum blends of 100:0, 95:5, 90:10, 85:15 and 80:20 were prepared, using both 75 and 85% extraction rate sorghum flours.

Chemical characteristics: The samples were analysed for moisture, total ash, dry gluten, sedimentation value, falling number and damaged starch content according to AACC (1983) procedures. Protein (Nx5.7) was estimated by micro-Kjeldahl method.

Rheological characteristics: The rheological characteristics of the composite flours were studied, using farinograph and extensograph according to the standard procedures (AACC 1983; Venkateswara Rao and Haridas Rao 1993).

Preparation and evaluation of bread: Breads were prepared with wheat and sorghum blends according to remix procedure with a reduced fermentation time of 120 min. for the dough, instead of 165 min. Evaluation of breads was carried out for crust and crumb characteristics after 24h of preparation by a panel of 6 judges

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TABLE 1. CHEMICAL*, RHEOLOGICAL AND BREAD MAKING CHARACTERISTICS OF WHEAT AND SORGHUM FLOUR BLENDS

	Wheat flour	Sorghum flour in blend (ER**75%) 5	10	15	20	Sorghum flour in blend (ER**85%) 5	10	15	20	SEM (df=27)
Chemical characteristics										
Ash, %	0.54^{e}	0.57^{d}	0.58^{d}	0.61^{c}	0.65^{b}	0.57^{d}	0.58^{d}	0.68^{d}	0.75^{a}	±0.008
Dry glutein, %	9.0^{a}	8.2^{b}	7.7^{c}	7.4^{d}	7.4^{d}	7.7	7.4^{d}	7.3^{e}	7.3^{f}	±0.32
Falling number, sec	432^{d}	437^{d}	460^{a}	459^{a}	448^{b}	438^{c}	440^{c}	453^{b}	460^{a}	±2.03
SDS Sedimentation value, ml	37^{a}	35^{b}	34^{b}	33^{b,c}	33^{b,c,d}	32^{d,e}	32^{d,e}	31^{e}	30^{f}	±0.51
Damaged starch, %	13.4^{a}	13.1^{b}	11.8^{c}	11.4^{d}	11.0^{e}	11.8^{c}	11.4^{d}	10.5^{f}	10.2^{g}	±0.27
Protein (Nx5.7),%	9.6^{a}	9.3^{b}	9.2^{d}	8.8^{f}	8.6^{g}	9.4^{b}	9.3^{c}	9.1^{d}	9.0^{e}	±0.02
Dough characteristics										
Farinograph										
Water absorption,%	62.1	61.8	61.9	61.3	60.6	60.2	59.7	58.4	57.7	–
Dough development time, min	2.0	2.5	2.5	2.5	2.5	1.0	1.0	2.0	2.0	–
Dough stability, min	5.5	5.5	5.0	5.0	5.0	5.5	5.0	5.5	5.5	–
Departure time, min	7.0	7.0	7.0	7.0	6.5	6.0	6.0	6.5	7.0	–
Mixing tolerance index at 10 min, BU	85	75	90	90	80	85	90	80	80	–
Valorimeter value	44	40	42	42	40	34	36	36	38	–
Extensograph										
Resistance to extension, R,BU	715	660	670	670	645	700	640	635	650	–
Extensibility, E, mm	134.5	136	137	111	107	136	122	112	96	–
R/E	5.31	4.63	4.89	6.03	6.03	5.14	5.08	5.67	6.78	–
Area, Cm^2	128.8	110	97.2	94.2	83.6	104	100	82.2	77.5	–
Bread characteristics										
Loaf weight, g	136.6^{f}	139.4^{b,c}	139.4^{b,c}	140.3^{b}	140.9^{a}	137.7^{c,f}	139.1^{d}	139.0^{d}	138.4^{e}	±0.39
Loaf volume, ml	535^{a}	505^{b}	460^{d}	430^{e}	420^{f}	480^{c}	420^{f}	410^{g}	375^{h}	±2.04
Specific loaf volume, ml/g	3.92^{a}	3.62^{b}	3.30^{d}	3.06^{e}	2.98^{f,g}	3.49^{c}	3.02^{f}	2.95^{f,g}	2.71^{h}	±0.02
Crumb colour	Creamish White	Dull White	Dull^{+} brown	Dull brown	Dull^{+++} brown	Dull^{+} White	Dull^{++} White	Dull^{++} brown	Dull^{+++} brown	–
Texture	Soft^{+++}	Soft^{++}	Soft^{++}	Slighlty hard	Hard	Soft^{+}	Slighlty hard	Hard^{+}	Hard^{++}	–
Grain	Fine Uniform	Fine Uniform	Fine Uniform	Medium fine Uniform	Medium fine Uniform	Medium fine Uniform	Medium fine Uniform	Medium fine Uniform	Medium fine Uniform	–
Score ***	7.5^{a}	7.0^{b}	7.0^{b}	6.5^{c}	6.5^{c}	6.5^{c}	6.5^{c}	6.5^{c}	6.0^{d}	±0.13

* Values expressed on 14% moisture basis

** Extraction rate

*** Maximum score 8

Means of the same column with different superscripts differ significantly ($P \leq 0.05$)

(Irvine and McMullan 1960). Loaf volume was measured, using rapeseed displacement method of Malloch and Cook (1930).

Chemical characteristics: The wheat flour had 9.6% protein, 9.0% gluten and sedimentation value of 37ml. The flour had low alpha amylase activity as shown by a falling number value of 432 (Table 1). The values showed that the flour was of medium strength quality, which fell in the range of typical values reported for Indian wheat flour by Shurpalekar et al (1976).

The data showed an increase in the ash content with the increase in the level of sorghum in blends. Negligible variation in alpha amylase activity was observed, as the falling number values ranged between 432 and 460. A decrease in gluten and sedimentation value was observed as the percent content of sorghum flour increased in the blend, indicating a decline in the quantity and quality of gluten (Table 1).

As the sorghum percentage increased in the blends, a decrease in percent damaged starch and protein was observed. The decrease in protein content of the blends could be attributed to the dilution of wheat protein with sorghum of lower protein level (Table 1).

Farinograph characteristics: A decrease in farinograph water absorption by 1.5 and 4.4% with 75 and 85% extraction rate refined sorghum flours, respectively on 20% substitution was noted.

Al-Mussali (1987) studied the effect of adding various amounts of sorghum flour to wheat flour on the quality of dough using farinograph, extensorgraph and maturograph and finished bread. Addition of 20% sorghum flour reduced the water absorption of the mixed flour. Dough development time remained unchanged at the 20% level. Studies carried out by Morad et al (1984), using farinograph and mixograph on the rheological properties of wheat -sorghum flour blends indicated that as the sorghum level increased, water absorption, peak time, time to breakdown and stability decreased, while mixing time increased.

The arrival time of 1.5 min for wheat flour varied from 1.5 to 2.0 min and 0.5 to 1.5 min with different levels of 75 and 85% extraction rate sorghum flours. There was marginal variation of 0.5 min in stability of dough with sorghum flour upto 20% addition. The dough consistency did not alter much even after 10 min mixing, as shown by maximum 10 BU variation in mixing tolerance index. The 85% extraction rate sorghum flour indicated more adverse effect on dough properties than 75% extraction rate flour, as shown by higher reduction in valorimeter value, which is an index of strength of the dough (Table 1).

Extensograph characteristics: The extensograph resistance to extension varied from 645 to 660 BU and 635 to 700 BU for wheat flour on substitution with different levels of 75 and 85% extraction rate refined sorghum flours, respectively. The extensibility decreased gradually with increase in sorghum content in the blend. Crabtree and Dendy (1977) prepared blends, which ranged in 5%, increments from wheat flour 100% to wheat 75/millet 25 and reported that as the proportion of millet increased, extensibility decreased.

The reductions in extensibility observed in this study were 27.5 and 38.5 mm on 20% replacement of wheat flour with 75 and 85% extraction rate sorghum flours, respectively. The ratio figure, which showed decrease till 10% addition, increased thereafter. The area value gradually decreased with increasing sorghum content in blend, indicating reduction in dough strength (Table 1). The adverse effect of sorghum flour on dough properties was larger with 85% extraction flour than with 75% extraction flour. Higher adverse effect of 85% extraction rate sorghum on dough properties could be attributed to the presence of more bran particles, which resulted in disruption of gluten films. Morad et al (1984) reported that the deterioration observed in rheological properties with increasing levels of sorghum in blends could be attributed to the dilution of wheat gluten upon sorghum substitution.

Bread making characteristics: There was a gradual decrease in loaf volume with the increasing sorghum content. The loaf volume decreased from 535ml of control to 420 and 375 ml for blends containing 75 and 85% extraction rate sorghum flours, respectively at 20% substitution (Table 1). The specific loaf volume also decreased by 0.94 and 1.21 ml/g (Table 1). Pringle et al (1969) described the production of bread from composite flours, using mechanical dough development. Bhatia et al (1968) stated that the mineral content in wheat and sorghum flour belnd bread was higher than that in wheat flour bread.

The crust colour and shape of the bread were unaffected. The colour of the crumb changed from creamish white to dull brown and intensity increased, as the sorghum content increased in the blend. Crabtree and Dendy (1977) also stated that

the bread colour darkened, as the proportion of millet increased.

In general, there was gradual hardening of crumb texture; as the addition of sorghum increased. The crumb score value reduced with the increase in sorghum content in the blend, indicating the adverse effect on crumb grain and texture and elasticity of crumb reduced with increase in sorghum content in blend. The crumb scoring was done as per the pattern followed by Patel (1992). At 20% addition of 75% extraction rate sorghum flour and more than 10% addition of 85% extraction rate sorghum flour a taste, not akin to bread was noticed in the breads (Table 1).

It may be concluded that substitution of wheat flour with sorghum flour to the extent of 15% and 10% in case of 75% and 85% extraction rate refined sorghum flour, respectively gives acceptable bread loaves.

References

AACC (1983) Approved Methods, 8th edn. American Association of Cereal Chemists, St. Paul, Minnesota, USA

Al-Mussali (1987) Pouziti smesnych psenicnych a cirokovych mouk pro peka-renskouvyrobu. Ph.D Thesis, University of Prague, Czechoslovakia

Badi SM, Hoseney RC, Casady AJ (1976) Pearl millet 1. Characterisation by SEM, amino acid analysis, lipid composition and prolamine solubility. Cereal Chem 53:478-487

Bhatia BS, Chakrabarty TK, Mathur VK, Siddaiah CH, Raghavan PKV (1968) Use of maize and milo flours in the preparation of bread. Indian Food Packer 22:33-35

Casey P, Lorenz K (1977) Millet-Functional and nutritional properties. Baker's Dig 51:45-47

Chavan JK, Kadam SS (1993) Nutritional enrichment of bakery products by supplementation with non-wheat flours. CRC Crit Rev Food Sci Nutri 33:189-226

Crabtree J, Dendy DAV (1977) Comilling of wheat and millet grain: A preliminary study. In: Proceedings of Symposium on Sorghum and Millets for Human Food. Tropical Products Institute, London, pp125-126

FAO Year Book-Production (1992) Vol. 46 pp 92-93

Haridas Rao P, Shurpalekar SR (1976) Utilisation of milo in bakery products. J Food Sci Technol 13:293-299

Hart MR, Graham RP, Gee M, Morgan Jr AI (1970) Bread from sorghum and barely flours. J Food Sci 35:661-665

Hulse JP, Laing EM, Pearson OE (1980) Sorghum and Millets; Their Composition and Nutritive value. Academic Press, UK p 404

Irvine GN, McMullan ME (1960) The remix baking test. Cereal Chem 37:140-152

Malloch TG, Cook WK (1930) A volume measuring apparatus for small loaves. Cereal Chem 7:307-310

Morad MM, Doherty CA, Rooney LW (1984) Effect of sorghum variety on baking properties of US conventional bread, Egyptian Pita Balady Bread, Cookies. J Food Sci 49:1070-1074

Patel MM (1992) Studies on the effect of blackgram (*Phaseolus mungo*) on rheological and baking characteristics of wheat flour Ph.D. Thesis, University of Mysore, Mysore, India

Pringle W, Williams A, Huke JH (1969) Mechanicallly developed doughs from composite flours. In: Composite Flour Programme. Documentation Package Vol.I, 2nd edn revised, Food and Agricultural Services Division, FAO, Rome, pp125-131

Samuel MA (1972) Bakery Technology and Engineering. 2nd edn. The AVI Publishing Co. INC, West port, Connecticut

Sumner AK, Nielsen MA (1976) Food Legume Utilization Progress Report #4, IDRC Project File #3, International Development Centre, Ottawa, Canada, p 73

Shurpalekar SR, Kumar GV, Venkateswara Rao G, Ranga Rao GCP, Rahim A, Vatsala CN (1976) Physico-chemical rheological, and mixing characteristics of bread and *chapati* making quality of Indian wheats. J Food Sci Technol 13:79-83

Venkateswara Rao G, Indrani D, Shurpalekar SR (1985) Effect of milling methods on the chemical, rheological and bread making characteristics of wheat flours. J Food Sci Technol 22(1):38-43

Venkateswara Rao G, Haridas Rao P (1993) Methods for determining rheological characteristics of doughs: A critical evaluation. J Food Sci Technol 30:77-91

Received 11 December 1995; revised 28 January 1997; accepted 3 February 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 3, 255-257

Acceptability and Qualities of *Boty Kababs* Prepared from Mutton and Rabbit Meat

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Mutton chunks were significantly low (P<0.01) in pH and protein content, but high in moisture, fat and ash contents as compared to rabbit meat. Reverse trends were observed for moisture and protein contents in cooked *Boty kababs*. Cooking yield was significantly low (P<0.01), while shear force values were significantly high (P<0.01) for mutton *Boty kababs*. Fresh rabbit *Boty kababs* were found more palatable than mutton. Storage for 10 days at 4±1°C did not affect the organoleptic characteristics, but microbial counts increased significantly (P<0.01). The study revealed that rabbit meat could be effectively used for preparation of *Boty kababs* of acceptable quality upto 10 days at 4±1°C.

Keywords: Mutton, Rabbit, *Kababs*, Proximate composition, Microbial qualities.

Besides being a prolific breeder and a source of high quality protein, rabbit has rapid growth rate, high fecundity and efficient feed conversion ration (Rao et al. 1978). Studies have shown that rabbit meat can be successfully used for frankfurters (Rao et al. 1979), sausages (Owen et al. 1985; Mendiratta and Panda 1992), *tandoori* and *kababs* (Kumar 1988). Most of the workers compared rabbit meat with chicken, pork and beef for different meat characteristics (Baker et al. 1972; Whiting and Jenkins 1981; Owen et al. 1985; Mendiratta and Panda 1992). Roy (1994) compared rabbit meat and mutton for sausage production. The objective of the present study was to compare the meats of two breeds of rabbit i.e., 'White Giant' and 'Soviet Chinchilla' with mutton for the production of a traditional ready-to-eat meat product *Boty kababs*.

Sheep (1 year old), 'White Giant' and 'Soviet Chinchilla' rabbits (both 12 weeks old) were slaughtered, deboned and kept at 4±1°C for 1–2 days before preparation of *Boty kababs*. Deboned chunks of meat were cut into pieces of about 40g each and marinated in solution (10% by weight), containing 1% citric acid and 2% aqueous salt solution for 30 min. Marinated meat pieces were slightly squeezed and re-marinated for 1 h with paste made up of 10% curd, 2% salt, 2.5% dried spices mixture and 4% wet condiments (onion 4 parts and garlic 1 part). Meat pieces wrapped with marinated paste were, then, put on skewers iron bar and roasted in gas tandoor at 170–190°C. The total roasting time was 20 min for rabbit and 50 min for mutton *kababs*. During roasting, the *kababs* were twice turned and sprinkled with vegetable oils. *Kababs* so prepared were divided into 3 parts, one part was evaluated fresh and other 2 parts were evaluated after 5 and 10 days storage at 4±1°C. Difference of weights of *Boty kababs* before and after roasting were also recorded for determination of cooking yields.

Moisture, protein, fat and ash contents of raw chunks and cooked *Boty kababs* were analysed as per AOAC (1980) methods. pH was determined with the help of ELICO pH meter. Warner Bratzler shear press (Model Chatillon USA) was used for determination of shear force values. Total plate counts, psychrotrophs, staphylococcus, coliforms and yeasts and moulds were determined as per standard procedures (Kumar 1988). Organoleptic evaluation was carried out by a panel comprising 15 semi-trained judges, using a 9-point Hedonic scale (Kumar 1988). Experiments were repeated thrice and data obtained were analysed statistically (Steel and Torrie 1960).

Raw chunks of 'White Giant' and 'Soviet Chinchilla' breeds of broiler rabbit were found to be significantly higher (P<0.01) for protein content, whereas fat and ash contents were significantly low (P<0.01) than mutton chunks (Table 1). These results are in agreement with the earlier studies (Rao et al. 1978; Roy 1994). Protein, fat and ash contents were higher in mutton *Boty kababs* but moisture content was lower than rabbit *Boty kababs*. This change in proximate composition is due to the shorter cooking time (20 min) required for rabbit *kabab* than for mutton *kababs* (50 min). This is also supported by lower cooking yield (P<0.01) of mutton *Boty kababs*. Mendiratta and Panda (1992) and Roy (1994) explained higher cooking yield in rabbit meat products due to higher water holding capacity.

pH values (Table 2) of raw mutton chunks were

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TABLE 1. PROXIMATE COMPOSITION OF RAW CHUNKS AND GRILLED *BOTY KABABS*

Species	Moisture, %		Protein, %		Fat, %		Ash, %		Cooking yield, %
	RC	BT	RC	BT	RC	BT	RC	BT	
Mutton	76.1±0.1^b	50.2±0.3^a	20.5±0.1^a	39.9±0.3^b	1.3±0.1^b	7.0±0.1^c	1.3±0.1^b	6.9±0.1^c	63.3±0.7^a
WG	75.1±0.1^a	57.7±0.2^b	21.9±0.2^b	30.2±0.1^a	1.0±0.1^a	6.2±0.1^b	1.0±0.1^a	5.3±0.1^a	75.5±0.5^b
SC	75.4±0.1^a	57.5±0.1^b	22.1±0.1^b	30.5±0.7^a	0.9±0.1^a	5.9±0.1^a	1.0±0.1^a	5.6±0.1^b	75.5±0.4^b
'F' value	13.2	16.9	29.8	108.0	51.7	57.0	9.9	131.1	166.6

Figures with same superscripts (parameterwise in a group) do not differ significantly ($P \leq 0.01$)
WG-White Giant, SC-Soviet Chinchilla, RC-Raw chunks, BT- *Boty kabab*

significantly lower (P<0.01) than those of rabbit chunks. Kumar (1988) also reported higher ultimate pH of rabbit meat due to insufficient glycogen content in rabbit muscles. pH values of *Boty kababs* were higher than their respective raw materials, but trends remain same. pH values were also not much affected during storage of the product. Shear force values (Table 2) of rabbit *Boty kababs* were significantly low (P<0.01) in comparison to mutton *Boty kababs* on all the three

TABLE 3. pH, SHEAR FORCE VALUE, MICROBIOLOGICAL QUALITIES AND ORGANOLEPTIC EVALUATION SCORES OF COOKED BOTY KABABS

Species	pH	shear force value kg/cm^2	Counts $\log_{10}$ cfu/g Total plate	Psychro-trophs	Staphylo-coccus	Coliform	Yeast and mould	Appear-ance	Colour	Odour	Taste	Tender ness	Juici-ness	Overall accep-tability
						Raw chunks								
Mutton	5.9±0.1^a	-	3.3±0.5	1.2±0.3^a	2.0±0.3	2.0±0.4	2.0±0.6	-	-	-	-	-	-	-
WG	6.1±0.1^b	-	4.0±0.2	2.9±0.2^b	2.5±0.2	2.0±0.2	1.1±0.5	-	-	-	-	-	-	-
SC	6.1±0.1^b	-	3.8±0.3	2.7±0.2^b	2.5±0.2	2.1±0.4	2.3±0.9	-	-	-	-	-	-	-
'F' value	11.44		NS	13.7	NS	NS	NS	-	-	-	-	-	-	-
						Boty kababs								
1st day of storage														
Mutton	6.1±0.1^a	2.3±0.1^b	2.4±0.4	1.8±0.2	1.0±0.4	1.5±0.2	1.4±0.2	6.4±0.3	7.3±0.2	7.3±0.2	6.8±0.4	6.6±0.4^a	6.5±0.4^a	6.7±0.3^a
WG	6.3±0.1^b	0.9±0.1^a	1.8±0.3	1.6±0.4	1.3±0.3	1.7±0.4	1.1±0.3	7.8±0.3	7.7±0.3	7.5±0.2	7.5±0.3	7.8±0.2^b	7.6±0.3^b	7.5±0.3^b
SC	6.3±0.1^b	0.9±0.1^a	2.1±0.5	1.2±0.5	1.2±0.4	1.7±0.9	1.6±0.4	7.8±0.3	7.8±0.3	7.6±0.2	7.7±0.3	7.8±0.3^b	7.7±0.2^b	7.9±0.3^b
'F' value	10.0	234.9	NS	NS	NS	NS	NS	NS	NS	NS	NS	5.1	4.2	4.3
5 days of storage														
Mutton	6.0±0.1^a	2.3±0.1^b	3.1±0.8	3.1±0.8	2.4±0.3	2.1±0.5	2.7±0.2	7.5±0.2	7.5±0.2	7.6±0.3	7.6±0.3	7.3±0.2	7.2±0.2	7.2±0.2
WG	6.2±0.1^b	0.8±0.1^a	3.5±0.2	3.0±0.7	1.6±0.5	2.1±0.3	2.4±0.4	7.9±0.2	7.7±0.2	7.5±0.2	7.5±0.2	7.5±0.2	7.7±0.2	7.6±0.2
SC	6.2±0.1^b	0.8±0.1^a	3.3±0.1	3.1±0.7	2.0±0.5	2.2±0.5	2.3±0.5	7.8±0.3	7.6±0.2	7.3±0.3	7.2±0.3	7.2±0.3	7.7±0.2	7.2±0.2
'F' value	12.3	83.7	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
10 days of storage														
Mutton	6.2±0.2	2.3±0.5^b	6.0±0.4	5.3±0.8	3.2±0.3	3.5±0.5	4.1±0.2	7.1±0.3	7.1±0.3	7.2±0.3	7.2±0.3	7.1±0.3	7.0±0.3	6.9±0.3
WG	6.3±0.1	0.8±0.4^a	5.8±0.6	5.6±0.6	2.9±0.4	3.2±0.7	4.0±0.3	7.4±0.1	7.3±0.2	6.8±0.3	6.8±0.2	6.9±0.3	6.9±0.2	6.8±0.3
SC	6.3±0.1	0.9±0.5^a	5.1±0.4	4.5±0.8	2.9±0.2	3.7±0.9	4.1±0.3	7.5±0.2	7.3±0.2	6.8±0.3	6.8±0.2	6.9±0.2	6.9±0.2	6.9±0.2
'F' value	NS	56.9	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interaction (Specieswise)														
Mutton	6.1±0.1^a	2.3±0.1^b	3.9±0.5	3.4±0.6	2.2±0.3	2.4±0.3	2.8±0.3	2.8±0.3	7.2±0.1^a	7.2±0.2	7.2±0.2	7.0±0.2	6.9±0.2^a	8.0±0.1
WG	6.3±0.1^b	0.9±0.1	3.7±0.5	3.4±0.6	2.0±0.4	2.5±0.4	7.7±0.1^b	7.5±0.1	7.5±0.1	7.3±0.1	7.3±0.1	7.4±0.1	7.4.01^b	7.3±0.1
SC	6.3±0.1^c	0.8±0.1^a	3.5±0.4	2.9±0.5	2.0±0.3	2.6±0.4	2.7±0.4	7.7±0.1^b	7.5±0.1	7.2±0.2	7.2±0.2	7.3±0.3	7.2±0.1^b	7.3±0.2
'F' value	12.1	103.3	NS	NS	NS	NS	NS	3.6	NS	NS	NS	NS	3.3	NS
Interaction (Storage period)														
Ist day	6.2±0.1^b	1.4±0.1^b	2.1±0.2a	1.5±0.2^a	1.2±0.2^a	1.4±0.3^a	1.4±0.2^a	7.5±0.2	7.5±0.2	7.5±0.1^b	7.3±0.2	7.4±0.3	7.3±0.2^{ab}	7.4±0.2^b
5 days	6.1±0.1^a	1.3+0.1^a	3.3±0.1^b	3.7±0.3^b	2.0±0.2^b	2.2±0.2^a	2.5±0.2^b	7.3±0.1	7.6±0.1	7.4±0.1^b	7.4±0.2	7.3±0.1	7.5±0.8^b	7.3±0.1^{ab}
10 days	6.3±0.1^b	1.3±0.1^{ab}	5.6±0.3^c	5.1±0.4c	3.0±0.2^c	3.5±0.3^b	4.1±0.1c	7.4±0.1	7.2±0.1	7.0±0.2^a	7.0±0.1	7.0±0.1	7.0+0.1^a	6.9±0.1^a
'F' value	3.77**	3.4**	66.9	23.2	17.8	13.3	53.3	NS	NS	3.9	NS	NS	3.7	3.3

Figures with same superscripts (parameterwise in a group) do not differ significantly (P/0.01)
** P/0.05, WG- White Giant, SC- Soviet Chinchilla NS: Not significant

storage intervals studied. This could be due to soft and fine muscle structure of rabbit meat (Roy 1994).

There were no significant differences for microbiological qualities (Table 2) of raw chunks of mutton and rabbit except psychrotrophic counts, which were significantly ($P<0.01$) high in chunks of two breeds of rabbit. This might be due to experimental variation, as rabbit chunks were kept for more time in refrigerator than mutton before preparation of *Boty kababs*. Three *Boty kababs* also did not differ significantly on day 1, 5 or 10 for microbial qualities. Microbiological counts of *kababs* increased significantly ($P<0.01$) from day 1 to day 5 or 10. The values in the present experiment are comparable to other reports on rabbit meat and meat products (Kumar 1988; Roy 1994).

Organoleptic evaluation (Table 2) revealed that freshly prepared *Boty kababs* of rabbit meat scored significantly higher ($P<0.05$) for tenderness, juiciness and overall acceptability than mutton *Boty kababs*. There was no significant difference between two breeds of rabbit. After 5 and 10 days of storage, all the three types of *kababs* scored almost equal for all the sensory attributes and there was no significant difference. Overall species-wise interaction showed significantly higher ($P<0.05$) scores for appearance and juiciness in rabbit kababs. From this, it is clear that *Boty kababs* prepared from rabbit had more desirable organoleptic qualities than mutton *Boty kababs*. Similar advantages of rabbit meat on sensory qualities of sausages were reported by Baker et al (1972) and Whiting and Jenkins (1981). There was not much effect of storage upto 5 days but after 10 days, there was significant ($P<0.05$) reduction in odour, juiciness and overall acceptability.

Based on these results, it can be concluded that both 'White Giant' and 'Soviet Chinchilla' breeds of broiler rabbit are promising alternate sources to mutton for production of ready-to-eat *Boty kababs*. The product prepared can be stored upto 10 days at refrigeration temperature without any adverse effect on sensory characteristics.

References

AOAC (1980) Official Methods of Analysis, 13th edn, Association of Official Analytical Chemists, Washington, DC

Baker RC, Darfler JM, Vadehra DV (1972) Acceptability of frankfurters made from chicken, rabbit, beef and pork. Poultry Sci. 51:1210-1214

Kumar S (1988) Studies on quality of rabbit meat, Ph.D. Thesis, University of Mysore, Mysore, India

Mendiratta SK, Panda PC (1992) Comparison of functional properties of chicken, pork and rabbit meat in different combinations in relation to yield while preparing sausage. Indian Vet J 69:241-245

Owen JE, Durate AOJ, Alarcon-Roja AD, Nunez Gonzalez FA, (1985) The substitution of rabbit meat for pork in the formulation of salichion sausages in Mexico. In:Proceedings of European Meet of Meat Research Workers 31:575-579

Rao DR, Chen CP, Sunki GR, Johnson WM (1978) Effect of weaning and slaughter ages on rabbit meat production. II. Carcass quality and composition. J Anim Sci 46:578-583

Rao DR, Chawan CB, Chen CP, Sunki GR (1979) Nutritive value of rabbit meat. In: Cheeke PR (ed), The Domestic Rabbit: Potential, Problems and Current Research. Proceedings of Symposium 71st Annual Meeting Amer Soc Anim Sci, USA, pp 53-59

Roy SK (1994) Studies on the quality of mutton sausages and rabbit meat + mutton combination sausages. M.V. Sc. Thesis, Indian Veterinary Research Institute, Izatnagar, India

Steel RGD, Torrie JH (1960) Principles and Procedures of Statistics. McGraw Hill Book Co. Inc., New York

Whiting RC, Jenkins RK (1981) Comparison of rabbit, beef and chicken meat for functional properties of frankfurter processing. J Food Sci 46:1693-1696

Received 23 November 1995; revised 28 January 1997; accepted 3 February 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 3, 258-260

Rheological Characteristics of Cane Juice During Sugar Manufacture

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The rheological characteristics of cane juice, including molasses and reboiling molasses, taken from different stages of sugar manufacture were determined, using a coaxial rotational viscometer within a temperature range of 30°C-90°C at an interval of 10°C. Using a Power Law Model of Shear-Stress- Shear Rate, flow behaviour index and consistency coefficient were calculated. The effect of temperature on the consistency coefficient was studied, using the Arrhenius equation. The activation energy of flow for the various samples ranged between 3.548 and 12.81 Kcal/g mole.

Keywords: Consistency coefficient, Flow behaviour index, Pseudoplastic, Rheology, Shear-stress, Sugarcane juice.

The manufacture of white consumable sugar from cane juice is carried out in various stages which, in addition, involve clarification, evaporation, crystalization and centrifugation. So, in the process of sugar manufacture, it is essential that complete information regarding properties of cane juice under various stages is made available. Since cane juice is a despersoid, consisting of materials of all degrees of dispersion (Payne 1964) like soil particles, bagasse particles, waxes, fats, proteins, gums, pectin, tannins, sugar, minerals and colouring matter. The rheological characteristics of the cane juice, thus, occupy an important place in estimating pumping requirements, rates of mixing, heat transfer, evaporation, filtration, crystallization, pan automation, power requirements of crystallizer and separation of sugar crystals. Therefore, the present study was undertaken to determine the rheological characteristics of cane juice at different stages of sugar manufacture and to study the effect of temperature on these rheological characteristics.

Various samples of cane juice, syrup, massicuites and reboiling and final molasses procured from various stages of sugar manufacture in Budhewal Cooperative Sugar Mills Ltd., Budhewal were anlysed to determine their °Brix, polarisation and purity. °Brix and polarisation values of the various samples were determined with the help of brix hydrometer and polarimeter, respectively using the procedure specified by Sugar Technologist's Association of India (Verma 1986). The purity of the juice was determined as the ratio of polarisation % to the Brix value (Table 1).

The rheological characteristics were measured in a rotational viscometer (Haake Rotoisco Model RV-1) in which the sample was introduced in the gap between a rotating and a fixed cylinder. The rotor rotated at different speeds to create different shear rate. Constant temperature water bath cum circulator (Universal thermostat type US) was used to maintain constant temperature (±0.02°C) during experimentation.

The shear-stress shear rate data obtained during experimentation is fitted to the Power Law Model (Heldman and Singh 1981) to determine the consistency coefficient (μ) and flow behaviour index (n).

The constitutive equation of this model is

$$\tau = \mu (- du/dy)^n$$

The slope of the resulting line indicates the flow behaviour index, while consistency coefficient is worked out by using the value of 'n' in the model at certain values of shear stress and corresponding shear rate (Fig. 1).

The Arrhenius equation is used to describe the influence of temperature on consistency coefficient (Fig. 2).

The equation is

$$\mu = A\ e^{B/T}$$

The regression coefficient between ln(μ) and B were calculated by fitting the data into an exponential curve using least square method.

Variation in consistency coefficient: Consistency coefficient of mixed juice, clarified juice and syrup showed smaller variation, whereas its concentration had increased from 14.36°Brix for mixed juice to 56°Brix for syrup (Table 1). In case of clarified juice, the consistency coefficient decreased as compared to mixed juice due to removal of various impurities such as muds, application of sulphur dioxide gas and addition of certain chemicals. Further, the addition of these chemical solutions

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TABLE 1. RHEOLOGICAL CONSTANTS OF POWER LAW MODEL

Temperature °C	°K	Consistency coefficient, Poise	Flow behaviour index	Activation energy (E), Kcal/g mole	R^2
Mixed juice (Bx = 14.36°, Purity = 82.38)					
30	303	0.4063	0.4063		
60	333	0.2139	0.6713	7.631	0.86
90	363	0.0763	0.7545		
Clarified juice (Bx = 15.19°, Purity = 82.55%)					
30	303	0.1468	0.7113		
60	333	0.0257	0.9201	9.359	0.85
90	363	0.1938	0.6228		
Syrup (Bx = 56°, Purity = 80.92%)					
30	303	0.3732	0.8868		
60	333	0.1314	0.9479	3.548	0.45
90	363	0.2119	0.8705		
A-Light (Bx = 68°, Purity = 83.39%)					
30	303	4.629	0.8661		
60	333	1.587	0.8162	7.538	0.93
90	363	0.572	0.7869		
Juice during charging of Pan-A (Bx = 72°, Purity = 87%)					
30	303	30.78	0.9214		
60	333	11.90	0.7578	8.076	0.93
90	363	3.00	0.8408		
C-Light (Bx = 80%, Purity = 55%)					
30	303	38.22	0.8491		
60	333	9.83	0.8293	5.381	0.88
90	363	8.55	0.7273		
A-Heavy (Bx = 84.7°, Purity = 68.48%)					
30	303	11.66	0.9011		
60	333	5.66	0.8087	5.514	0.90
90	363	2.29	0.8153		
B-Heavy (Bx = 90°, Purity = 46.5%)					
30	303	129.50	0.9159		
60	333	24.22	0.8112	8.73	0.96
90	363	10.61	0.6850		
Juice during start of Pan-C (Bx = 90°, Purity = 56%)					
40	313	323.27	0.9410		
60	333	77.63	0.9357	13.96	0.98
90	363	16.07	0.9047		
Final molasses (Bx = 90.1°, Purity = 28%)					
30	303	144.76	0.9630		
60	333	20.66	0.8627	9.448	0.90
90	363	13.86	0.7450		
A-Massicuite in crystalliser (Bx = 91.4°, Purity = 92%)					
30	303	250.73	0.8682		
60	333	131.22	0.8334	6.031	0.92
90	363	47.66	0.7502		
B-Massicuite in crystalliser (Bx = 94.92°, Purity = 68.5%)					
60	333	257.30	0.7864		
70	343	71.21	0.9348	19.81	0.92
90	363	24.23	0.9264		

during clarification as well as dosing of these chemical solutions in each of the bodies of multiple effect evaporator during evaporation to provide antiscaling effect had reduced the viscosity. Consequently, the syrup, which came out from the last effect of evaporator seemed to have little variation in consistency with temperature (Fig. 2).

In case of reboiling molasses i.e., A-light, A-heavy, B-heavy and C-light, the consistency coefficient increased from 4.629 poise at 30°C for A-light to 129.50 poise for B-heavy at the same temperature. However, C-light with lower Brix (80°) than A-heavy (84.7°) had higher consistency coefficient and this increase in consistency coefficient

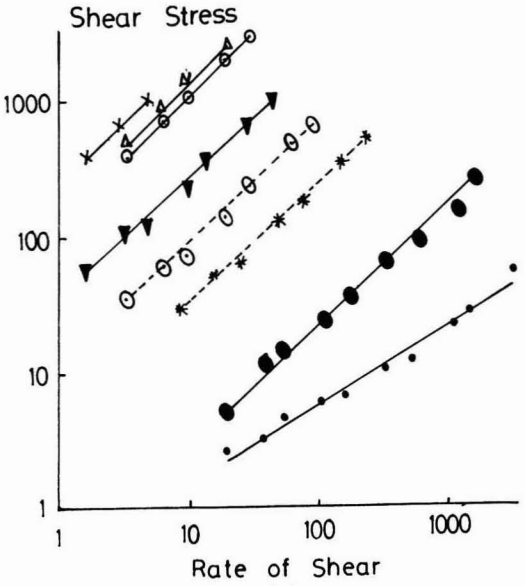

Fig 1. Shear stress Vs rate of shear at 30°C
-•- Mixed juice, -⊕- Syrup, --•-- A-light,
-⊙- A- Heavy, -⊙- B-Heavy, -▼- C-Light,
-Δ- Final molasses, -x- A Massicuite in cryst

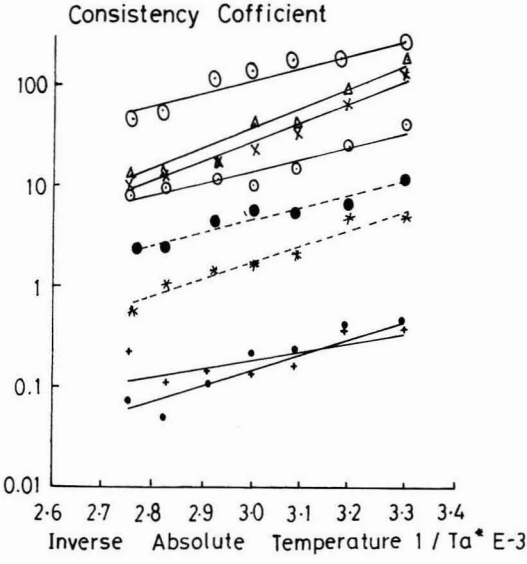

Fig 2. Effect of temperature on consistency coefficient
-•- Mixed juice, -+- Syrup, --•-- A-light,
-⊕- A- Heavy, -x- B-Heavy, -⊙- C-Light,
-Δ- Final molasses, -⊙- A Massicuite in cryst

may be due to the lower purity of C-light. So, in case of reboiling molasses, the consistency coefficient increased with decrease in the purity. Similarly, for final molasses having concentration of 90.1°Brix and purity of 28%, the consistency coefficient varied from 144.76 poise at 30°C to 13.86 poise at 90°C.The coefficient of determination (R^2) for each of the samples was found to vary between 0.94 and 0.99.

Variations in flow behaviour index: The flow behaviour index of cane juice drawn from different stages of sugar manufacture was less than unity, which indicated the pseudoplastic nature of the juice. However, there did not appear to be larger variations among the values of 'n' at different temperatures for all the cases.

In case of reboiling molasses, the 'n' values tended to decrease slightly with increase of temperature (Table 1). For the rest of the cases, the flow behaviour intended to increase with rise of temperature. The 'n' values were found to vary between 0.5751 and 0.9715.

Effect of temperature on rheological parameter: The Arrhenius equation fitted well to describe the temperature influence on all the samples of cane juice considered (Fig. 2). The flow behaviour index did not change significantly, indicating pseudoplastic flow at all the temperatures. The activation energy of flow (E) in case of mixed juice was found to be 7.631 kcal/g mole and 12.27 kcal/g mole for C-massicuite in crystaliser. However, the minimum value of activation energy was observed for syrup, whereas the maximum value was for B-massicuite in crystaliser. No specific relationship of activation energy with concentration and purity of juices could be seen.

Notation

τ	=	Shear stress (Pa)
du/dy	=	Rate of shear (sec^{-1})
μ	=	Consistency coefficient (Poise)
E	=	Activation energy (Kcal/g mole)
R	=	Universal gas constant, (Kcal/kg mole)
A and B	=	Constant

References

Heldman DR, Singh RP (1981) Food Process Engineering. The AVI Publishing Co. Inc., West Port

Payne JH (1964) Fundamental reactions of the clarification process. In: Honig P (ed), Principles of Sugar Technology, Vol I. Elsevier Publ. Co., New York, p 502

Verma NC (1986) Systems of Technical Control for Cane Sugar Factories in India. Published by The Sugar Technologists' Association of India, Kanpur

Received 18 May 1996; revised 28 January 1997; accepted 4 February 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 3, 261-263

Development of a Bovine Plasma Medium for Propagation of Lactobacilli

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Bovine plasma medium (BPM), based on bovine blood plasma that can be heat-sterilized, is described. Bovine plasma solution (BPS) was prepared by mixing 300 ml of bovine plasma with 300 ml of distilled water. The solution was adjusted to pH 11 and was sterilized in an autoclave at 121°C for 15 min. The sterile BPS was then mixed with a sterile solution of glucose, minerals and yeast extract. The final pH after mixing was about 6.4. The individual effects of minerals and yeast extract were also tested. The microorganisms used to test the medium were *L. Oplantarum, L. casei, L. bulgaricus* and *L. acidophilus*. The efficiency of the new medium was compared with commercial MRS and no differences in the growth of the different *Lactobacilli* were observed. It was concluded that a new, low cost, practical medium could be developed for the propagation of *Lactobacilli*.

Keywords: Bovine plasma medium, *Lactobacilli*, pH, Heat-sterilization, Propagation.

The use of selected *Lactobacilli* as food supplements is on the increase, as consumers have recognized the desirability of fermented food and the contribution of certain intestinal *Lactobacilli* to human wellbeing (Speck et al. 1993).

Strains of *Lactobacilli* are used as starter cultures for dairy, meat and vegetable products (Classy 1985).

Many media have been prepared over the years for *Lactobacilli* (Briggs 1953; Evans and Niven 1951; Deibel et al. 1957). An improved medium for *Lactobacilli* cultivation was developed by De Man et al (1960).

Tybor et al (1975) have reported that bovine plasma is a source of large quantities of dietary proteins and contains all of the essential amino acids. *Lactobacillus* grows well, when plasma albumin is used to enrich the medium (Briggs 1953). However, use of bovine plasma was discontinued because it was not possible to sterilize it by autoclaving. Barboza et al (1994) used bovine plasma to formulate a medium for *Lactobacillus*. But, this had to be sterilized by Seitz-Filtration due to the tendency of the medium to coagulate even at low concentration of bovine plasma.

In developing countries, to obtain media for *Lactobacillus* propagation is rather difficult and costly. This has created the need to look for other alternatives. The purpose of this study was to evaluate the use of bovine plasma as a source of protein in the formulation of a *Lactobacillus* culture medium that can be heat-sterilized.

Blood collection: Plasma was obtained from bovine blood by centrifugation at room temperature and frozen at -20°C, until used.

Bacterial strains: The microorganisms used in this study were *Lactobacillus plantarum* ATCC 8014, *Lactobacillus casei* ATCC 7469, *Lactobacillus bulgaricus* ATCC 11842, and *Lactobacillus acidophilus* ATCC 4356. The cultures were maintained by routine subcultures in *Lactobacilli* MRS broth obtained from Merck (D-61 Darmstadt), using 1% inocula and 18 h of incubation at 37°C in a Gas Pak Jar with 10% CO_2 and were refrigerated (5°C) between transfer. Test cultures were transferred at least three times before being used experimentally.

Heat sterilization of the plasma: Bovine plasma(300 ml) were diluted to 1000 ml with distilled water. The solution was divided into 4 portions. The pH of the first portion was adjusted to 7.4, the second to 9, the third to 11. In all the cases, 1N NaOH was used to adjust the pH. Then, all the portions were autoclaved at 121°C for 15 min. The portion with the highest pH which remained a clear liquid without any turbidity after heat sterilization, was selected as a component in the formulation of the medium.

Preparation of the bovine plsma medium (BPM): Bovine plasma solution (BPS) was prepared by mixing 300 ml of bovine plasma with 300 ml of distilled water, pH was adjusted to 11 and sterilized in an autoclave at 121°C for 15 min. A solution of glucose, minerals and yeast extract (GMY) was prepared by dissolving 10 g of glucose, 6 g of sodium acetate, 1 g of ammonium citrate, 3 g of KH_2PO_4, 0.05 g of $MnSO_4$ H_2O, 0.5 g of $MgSO_4$ H_2O and 5 g of yeast extract (Difco Laboratories, Detroit Michigan USA) in 400 ml of distilled water, mixed

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and heated with frequently stirring, until complete dissolution and then sterilized in an autoclave at 121°C for 15 min. To make BPM, 600 ml of sterile BPS was mixed with 400 ml of sterile GMY solution. Addition of the GMY solution lowered the pH to 4.6 approximately and left the medium ready for use.

BPM agar: BPM agar was prepared by adding 20 g of agar to the 400 ml GMY solution, mixed and boiled with frequently stirring, until complete dissolution, sterilized and mixed immediately with sterile BPS solution. It was allowed to stand until the temperature reached 50°C and dispensed aseptically in sterile plates. The complete medium did not remelt.

Protein and amino acid analysis of the medium: Protein was analyzed by the Kjeldahl procedure (AOAC 1980). Amino acids were analyzed by High-Performance Liquid Chromatography. A Shimadzu model LC6A HPLC equipped with a FLD6A Fluorescence detector, two LC6A pumps, a SCL-6B auto injector, CTO-6A column oven and a C-R4A Chromatopack, integrator was used throughout the experiments. An Altex ultrasphere ODS, C-18, 15 cm length x 4 mm ID, 5 µm column was used.

Two solvent systems were used. Solvent A composed of acetate buffer (0.05 M), methanol and tetrahydrofurane (80.19:1). Solvent B composed of methanol and acetate buffer (80:20). A Sigma Lab standard solution 50nmol/ml amino acid concentrations was used as a reference. Pre-column derivatization of the amino acids was performed. Samples of 20 µl were injected onto the column. Flow rate was 1 ml/min. Fluorescence was read at 470 nm with an excitation wave length of 350 nm. Peak areas were used for quantitative calculations.

To test the individual effect of minerals and yeast extract, 600 ml of BPS were mixed with either 400 ml of a sterilized solution of glucose and potassium diphosphate (potassium diphosphate was added to bring down the pH to around 7.0), or 400 ml of a sterilized solution of glucose, phosphate and minerals, or 400 ml of a sterilized solution of glucose, phosphate and yeast extract. The amount of glucose, diphosphate, minerals and yeast extract added to the 400 ml of each of the sterilized solutions was the same as used for the preparation of the GMY solution.

Microorganisms were grown in each medium for 18 h at 37°C in a Gas Pak jar with 10% CO_2 and counts of *Lactobacilli* were compared on MRS agar and BPM agar. *Lactobacilli* cell numbers in growth media were estimated by plate count surface or spread method on MRS agar and BPM agar. Plates were incubated at 37°C for 48 h in a Gas Pak jar with 10% CO_2. Results were expressed as $\log_{10}$ colony-forming units per ml (cfu/ml).

TABLE 1. AMINO ACID CONTENTS OF THE BPM MEDIUM

Amino acids	Mg/ml of medium	%*
Aspartic acid	3.20	12.06
Glutamic acid	3.99	15.04
Histidine	1.05	3.95
Serine	1.62	6.10
Glycine	1.43	5.39
Threonine	1.93	7.27
Arginine	2.00	7.54
Alanine	1.93	7.27
Tyrosine	1.29	4.86
Methionine	0.34	1.28
Valine	2.09	7.88
Phenylalanine	1.65	6.22
Isoleucine	0.64	2.41
Leucine	2.50	9.42
Lysine	2.86	10.78

*Percentages of the total amount of amino acids

The efficiency of the BPM as a culture medium was compared against a MRS commercial medium. pHs of the cultures were determined at 25°C, using a Metrohm 620 pH meter, after 18 h of incubation.

The appearance of BPS at all pH values (7.4, 9.0, 10 and 11.0) before sterilization was normal. After heat sterilization, the degree of turbidity of BPS decreased, as the pH increased from 7.4 to 11.0. The BPS at pH 7.4 coagulated on sterilization, while at pH 10.0 it turned milky and turbid, respectively. The BPS at pH 11 did not change its appearance under heat sterilization conditions. These results are of utmost importance, because one of the limitations for using bovine plasma as a culture medium is its tendency to form gels, when subjected to heat sterilization.

Amino acid contents of the BPM expressed either as mg of amino acids per ml of medium or as percentage of the total amount of amino acids are presented in Table 1. Results have shown that bovine plasma medium formulated in this study contains all the amino acids required by most species of *Lactobacillus* that have so far been examined (Morishita et al. 1981). Total protein concentration of the BPM was 3.27.

Results of average growth values for *Lactobacilli* in the MRS, BPM and its different variations are summarized in Table 2. No difference in *Lactobacilli* growth in the BPM and MRS medium was

TABLE 2. MEAN VALUES *FOR *LACTOBACILLI* GROWTH IN MRS, BPM AND ITS DIFFERENT VARIATIONS

			Variations**		
Microorganisms	MRS	BPM	A	B	C
L. plantarum	8.49[a]	8.40[a]	8.29[b]	7.55[c]	7.44[c]
L. casei	7.71[a]	7.69[a]	7.25[b]	7.35[b]	7.23[c]
L. acidophilus	8.07[a]	8.01[a]	7.80[b]	7.56[c]	7.27[d]
L. bulgaricus	7.60[a]	7.56[a]	7.40[a]	7.10[b]	7.01[b]

*Log_{10} cfu/ml

**A=BPM without the addition of yeast extract,
B= BPM without the addition of minerals, C= BPM without the addition of minerals and yeast extract.

MRS= Man, Rogosa and Sharpe(1960) Medium

BPM= Bovine Plasma Medium

[a,b,c,d] Means on a row bearing different superscripts differ significantly ($P<0.05$).

observed. pH reduction after 18 h was between 4.1 and 4.2 in both media. Mineral supplementation was more efficient than yeast extract.

The lower but still remarkable bacterial growth was observed, when BPM without the addition of minerals and yeast extract was used, indicating that a less expensive simple growth medium based on bovine blood plasma can be used for *Lactobacilli* propagation.

The authors acknowledge with thanks the financial support extended by CONDES and PTU.

References

AOAC (1980) Official Methods of Analysis. 13th edn. Association of Official Analytical Chemists, Washington, DC

Barboza Y, Marquez E, Arias B, Faria J, Castejon O (1994) Utilization de plasma sanguineo de bovino como fuente proteica en la formulacion de un medio de cultivo para lactobacilos. Revista Cientifica FCV 1:55-59

Briggs M (1953) An improved medium for *Lactobacilli*. J Dairy Research 20:36-39

Chassy B (1985) Prospects for improving economically significant *Lactobacillus* strains by genetic technology. Trends in Biotechnology 3:273-275

De Man J, Rogosa M, Sharpe M (1960) A medium for the cultivation of *Lactobacilli*. J Applied Bacteriol 23:130-135

Deibel R, Evans J, Nieven C (1957) Microbiological assay for thiamine using *Lactobacillus viridescens*. J Bact 74:818-821

Evans J, Niven Jr C (1951) Nutrition of the heterofermentative *Lactobacilli* that cause greening of cured meat products. J Bacteriol 62:599-603

Morishita T, Deguchi Y, Yajima M, Sakurai T, Yura T (1981) Multiple nutritional requirements of *Lactobacilli*. Genetic lesions affecting amino acid biosynthetic pathways J Bacteriol 120:1078-1084

Speck M, Dubroguszw J, Casas I (1993) *Lactobacillus reuteri* in food supplementation. Food Technol 7:90-94

Tybor P, Dill C, Landmann W (1975) Functional properties of proteins isolated bovine blood by a continuous pilot process. J Food Sci 40:155-158

Received 17 March 1995; revised 28 January 1997; accepted 6 February 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 3, 264-266

Influence of Calcium on the Toxic Effects of Dietary Aluminium

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Aluminium chloride ingestion at a dose of 50mg/kg body wt/day for 40 days caused toxic effect on rat liver and kidney, as was revealed by the histopathological observations and acid and alkaline phosphatase activities in liver, kidney and blood. The toxic effect on kidney was less than that on liver. Simultaneous oral administration of calcium chloride at 50, 100, 200 and 400 ppm concentration daily in drinking water could overcome the toxic effect of aluminium. Calcium (50 ppm) could normalise the acid and alkaline phosphatase activities and also the histopathological changes in liver.

Keywords: Aluminium, Liver, Kidney, Toxicity, Calcium.

Aluminium (Al) is commonly used in food processing, storage, pharmaceuticals and as phosphate binders. Human being is accumulating Al from processed cheese, which contains 297μg/g Al, whereas natural cheese (country cheese) contains 15.7 μg/g Al. The most remarkable are bakery products, because baking powder contains 23000 μg/g Al (in the form of Na-Al-phosphate). Cocoa contains 45μg/g and pickles with Al additives (Aluminium ammonium sulphate) contains 40μg/g. There are salts with Al additives (164μg/g). Tea packed in bags contains 1280μg/g, whereas steeped tea contains only 5μg/g. Milk addition to tea reduces Al absorption, whereas lemon addition increases this. Again, Al cooking pans also adds Al to food. Increases in Al contents in beef roast, cabbage and tomato sauce cooked in Al pan have been found to be about 4.5 times, 28 times and 571 times, respectively. Moreover, tinned foods, foods decorated with Al foil instead of silver foil, antacids, some pain killers are potential sources of Al (Greger 1988). Al is deposited in liver, kidney brain and bone (Greger 1988). Due to Al deposition in brain, plaques are formed and Alzheimer's type presenile dementia may arise (Peri 1984). Deposition of Al in bone results in angular deformities, osteoporosis, progressive fracturing and osteodystrophy (Mehls and Salusky 1987). A number of amytrophic lateral sclerosis patients were reported from Guam and Kii and it was reported that their drinking water contained less Al and high calcium (Ca) (Peri 1984). Furthermore, it has been reported by Chaudhuri and Chaudhuri (1995) that presence of Ca in drinking water has some protective role against Al toxicity. The aim of the present study was to find out a critical concentration of Ca in drinking water, which might have the said protective role.

Animal experiment: Male albino rats (Sprague Dawley strain) of average body weight (160 g) were caged (cages painted with Al paint) with *ad libitum* food and water in 12 h light-12h dark cycle. Rats were divided into 6 groups. Group 1 was given distilled water for drinking and this group was considered as control group. Group 2 rats were given orally $AlCl_3$ with distilled water (50 mg/kg body weight/day, maximum safe dose recommended by Yokel and McNamara 1985) for 40 days. The pH of the solution was maintained at 3 to avoid precipitation of Al, as $Al(CH)_3$. Groups 0, 3, 4, 5, 6 were treated similarly with $AlCl_3$ and 50, 100, 200 and 400 ppm Ca, respectively as $CaCl_2$.

Collection of tissues: After 40 days of treatment, rats were sacrificed and liver, kidney and exsanguinated blood were collected at 0°C.

Enzyme assay : Tissues were homogenised (10%) in saline. Homogenates were centrifuged at 10,000 g for 10 min. and the supernatants were used as the source of the enzymes, acid phosphatase and alkaline phosphatase. Enzymes were assayed following the method of de Duve et al (1955) using Na-β-glycero-phosphate as substrate and Na-acetate-acetic acid as buffer (pH 5) and glycine-NaOH as buffer (pH 9) for acid phosphatase and alkaline phosphatase, respectively. Protein was determined following the method of Lowry et al (1951). Blood free phosphate was determined using ammonium molybdate and ascorbic acid (de Duve et al. 1955).

Histological studies: Liver and kidney tissues were fixed in 10% formaldehyde-saline and slides

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TABLE 1. ACID AND ALKALINE PHOSPHATASE ACTIVITIES IN TISSUES OF $AlCl_3$ (50 mg/kg body wt./day) oral-TREATED RATS.

Tissue		Control animals	$AlCl_3$ treated animals			
			No Ca^{2+}	50 ppm Ca^{2+}	100 ppm Ca^{2+}	200 ppm Ca^{2+}	400 ppm^{2+}
Liver	Acid phosphatase, µgpi/mgpr./h	3.42 ±0.16	6.12**±0.57	4.8⁺ ±0.45	4.56⁺ ±0.41	5.63**±0.19	2.55 ±0.28
	Alkaline phosphatase, µgpi/mgpr./h	1.54 ±0.13	3.45⁺⁺ ±0.15	1.58 ±0.09	2.38⁺⁺ ±0.14	2.39* ±0.12	2.64 ±0.43
Kidney	Acid phosphatase, µgpi/mgpr./h	6.44 ±0.89	6.21 ±0.94	6.23 ±1.03	5.93 ±1.21	7.13 ±0.86	6.92 ±0.50
	Alkaline phosphatase, µgpi/mgpr/h	15.80 ±1.31	21.75⁺⁺ ±1.42	14.31 ±1.90	21.05⁺⁺ ±2.18	18.36 ±2.37	22.51⁺ ±1.51
Blood	Free phosphate, mgpi/100 ml blood	29.99 ±1.12	30.07 ±2.11	29.99 ±2.05	21.90⁺⁺ ±1.67	25.94 ±3.02	22.14⁺ ±2.26
	Alkaline phosphatase, µgpi/100 ml blood	19.82 ±1.86	13.01 ±1.16	12.28⁺±0.64	67.91**±4.96	10.18 ±1.73	10.6⁺ ±1.71

Results are expressed in ± SEM of 8 rats.

⁺ $P < 0.05$; ⁺⁺ $P < 0.02$ * $P < 0.01$ ** $P < 0.001$

One way Anova calculation data give identical significance and non-significance like students' 't' test taking one enzyme and one tissue at a time

were stained with eosin and haematoxylin.

From Table 1, it is evident that liver acid phosphatase was increased by more than two fold ($p<0.01$), as a result of $AlCl_3$ (50 mg/kg body weight/day) oral treatment for 40 days, indicating liver toxicity. The acid phosphatase activity was reduced (20%), when drinking water contained 50 ppm of Ca. Addition of 100 ppm Ca in drinking water brought the acid phosphatase activity to normal level.

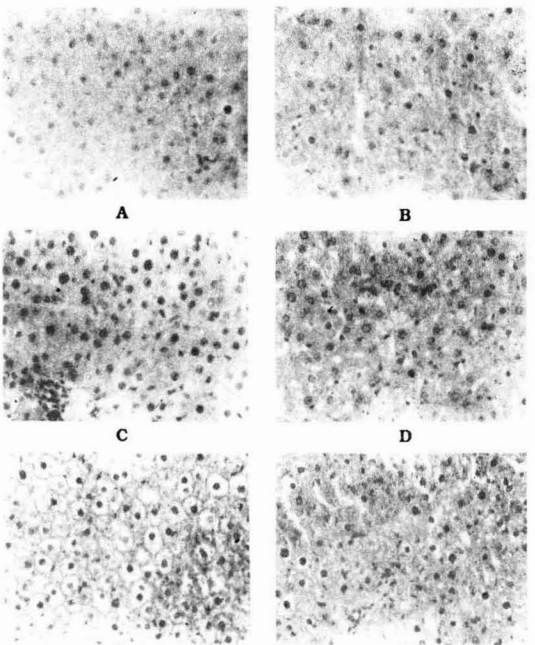

Fig. 1. Histopathological observations of liver slices (5 microns) x 250 of rats treated for 40 days orally with $AlCl_3$ (50mg/kg body wt/day) in presence and absence of $CaCl_2$ in drinking water. Eosin-haematoxylin stain. A. control rat; B. only $AlCl_3$ treated; C. $AlCl_3$ treated rats getting 50 ppm of Ca^{2+}; D. $AlCl_3$ treated rats getting 100 ppm Ca^{2+}; E. $AlCl_3$ treated rats getting 200 ppm Ca^{2+}; F. $AlCl_3$ treated rats getting 400 ppm Ca^{2+}.

Liver alkaline phosphatase activity increased (Table 1) significantly ($p<0.02$) due to Al treatment. Calcium (50 ppm) in drinking water could restore the activity to normal level.

Histopathological studies revealed that $AlCl_3$ treatment resulted in nuclear hypertrophy and lipid accumulation in liver (Fig. 1B), whereas no lipid accumulation was found in rats, which were getting 50 ppm Ca in drinking water (Fig. 1C). In these tissues, individual cells and cell membranes were easily recognisable. Vacuoles were not found. However, cellular hypertrophy was evident. Calcium at a concentration of 100 ppm developed nuclear hypertrophy (Fig. 1D). Individual cell membranes were no longer visible and the presence of numerous small nuclei were observed. In case of group 5 rats, the presence of numerous nuclei were found and hyperplasia was also observed (Fig. 1E). Cytoplasmic degranulation was observed in hepatocytes of group 6 rats (Fig. 1F). The cells were not clearly distinguishable, nuclear outlines were no longer round and partially crumpled with increasing staining intensity. Large healthy nuclei were less abundant in comparison to smaller nuclei. Therefore, it appears that presence of at least 50 ppm of Calcium in drinking water could effectively decrease the toxic effect of Al on rat liver. Calcium itself at 200 and 400 ppm concentrations could not affect the enzyme activities.

In contrast to liver, no significant change in kidney acid phosphatase acitivity was found due

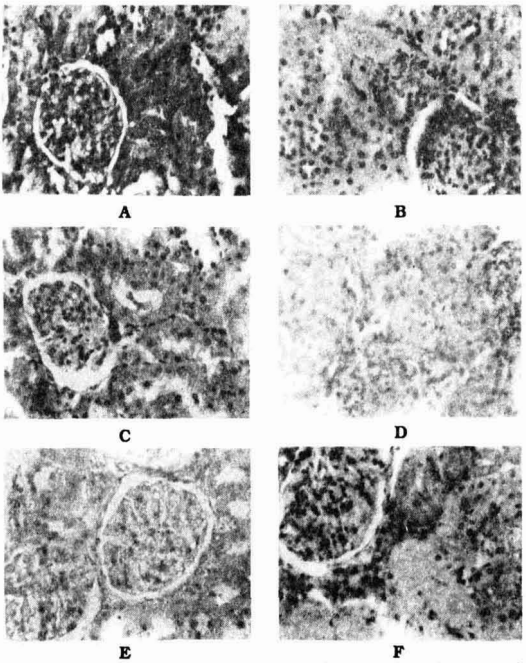

Fig. 2. Histopathological observations of kidney slices (5 microns) x 250 of rats treated for 40 days orally with $AlCl_3$ (50mg/kg body wt/day) in presence and absence of $CaCl_2$ in drinking water. Eosin-haematoxylin stain. A. control rat; B. only $AlCl_3$ treated; C. $AlCl_3$ treated rats getting 50 ppm of Ca^{2+}; D. $AlCl_3$ treated rats getting 100 ppm Ca^{2+}; E. $AlCl_3$ treated rats getting 200ppm Ca^{2+}; F. $AlCl_3$ treated rats getting 400 ppm Ca^{2+}.

to $AlCl_3$ treatment. On the other hand, kidney alkaline phosphatase activity was increased ($p<0.02$) due to $AlCl_3$ treatment and the activity became almost normal, when 50 ppm Ca was present in drinking water.

Histopathological studies revealed that due to $AlCl_3$ treatment, the nuclei in the glomeruli became prominent and deeply stained and in few glomeruli, presence of vacuoles was observed (Fig. 2B). In the glomeruli of group 3 rats, the presence of numerous vacuoles was found (Fig. 2C). Nuclei were of various shapes and sizes. Tubular size appears to be enlarged with smaller lumen. Nuclei numbers were also increased. Increase in the nuclear number and tubular size was also observed in group 4 rats (Fig. 2D). In rats getting 200 ppm Ca in drinking water along with $AlCl_3$, the nuclei numbers in the glomeruli were less and network was less intensely stained. A vacuolar appearance was also observed (Fig. 2E). The serum alkaline phosphatase activity was decreased during $AlCl_3$ intoxication (Table 1). In presence of both Al and Ca in drinking water, there was no significant change in the activity of the enzyme. At a 100 ppm concentration of Ca, the alkaline phosphatase activity was found to increase significantly.

The literature indicates that i) Plasma alkaline phosphatase was usually low in patients treated with Al during dialysis (Mehls and Salusky 1987), ii) Aluminium was found to inhibit the bone alkaline phosphatase activity at a concentration above 1.5 μM (Lieberherr et al. 1982) and iii) Alkaline phosphatase activity in culture of osteoblast like cells was inhibited in the presence of Al (Lieberherr et al. 1987).

Free phosphate level remained almost unaltered in the presence of Al only and also in presence of Al and 50 ppm Ça in drinking water.From this study, it can be concluded that an optimum critical concentration of Ca may be supplemented with the processed foods so that Al toxicity may be decreased.

References

Chaudhuri AN, Chaudhuri D (1995) Aluminium-calcium interaction in rat brain, liver and kidney. In: Abdulla M, Vohara SB, Athar M (eds), Trace and Toxic Elements in Nutrition and Health, Jamia Hamdard and Willey Eastern Ltd. New Delhi, pp 60-66

de Duve C, Prenman CBC, Sianetto R, Wattiaux R, Appleman F (1955) Intracellular distribution patterns of enzymes in rat liver tissue. Biochem J 60:604-617

Greger JL (1988) Aluminium in the diet and mineral metabolism In: Sigel H, Sigel A (eds) Metal Ions in Biological Systems: Aluminium and Its Role in Biology, Marcel Dekker Inc. New York, pp 199-216

Lieberherr M, Groose B, Carnot Winter C, Thill CL, Balsan S (1982) *In vitro* effects of aluminium on bone phosphatases: A possible interaction between parathyroid hormone and vitamin D_3 metabolites. Calcif Tissue Int 34(3):280-284

Lieberherr M, Grosse B, Cournot Winter C, Hermann-Erlee MPM, Balsan S (1987) Aluminium action on mouse bone cell metabolism and response to parathyroid hormone and 1, 25 $(OH)_2D_3$. Kidney Int 31(3):736-743

Lowry OH, Rosebrough NJ, Farr AL, Randal RJ (1951) Protein measurement with Folin-phenol reagent. J Biol Chem 193:265-275

Mehls O, Salusky IB (1987) Recent advances and controversies in childhood renal osteodystrophy. Ann Intern Med 101:775-780

Peri DP (1984) Aluminium and Alzheimer's disease: Methodologic approaches in Metal ions. In: Sigel H, Sigel A (eds) Metal Ions in Biological Systems: Aluminium and Its Role in Biology, Marcel Dekker Inc. New York, pp 259-279

Yokel RA, MacNamara PJ (1985) Influence of renal impairment chemical form serum protein binding on intravenous and oral aluminium kinetics in rabbits. Toxicol Appl Pharmacol 95:32-43

Received 24 October 1995; revised 4 February 1997; accepted 6 February 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 3, 267-269

Dye Reduction Test to Assess the Bacteriological Quality of Fish Stored in Ice

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The bacteriological load of fishes stored in ice was evaluated by the reduction time of resazurin and methylene blue dyes. An inverse relationship existed between aerobic mesophilic counts and dye reduction times. Results have indicated that dye reduction test can be used as a screening test in rapidly determining the bacterial load of fishes stored under iced condition. Very highly significant ($p<0.01$) negative correlations ($r \geq 0.969$) was obtained for both the dyes used in relation to microbial load of all the samples.

Keywords: Aerobic mesophilic count, Resazurin, Methylene blue, Dye reduction times, Sensory evaluation, Fishes.

Aerobic bacterial count is an important parameter used in determining the bacteriological quality of fishes. The conventional bacterial counting procedures are time consuming, which may delay the effective control of quality of fish and fishery products. Presently, India is earning about $1000 millions as foreign exchange through the export of seafoods. The export of good quality seafoods on a sustainable basis demands an effective quality control system, whereby the quality of the raw materials must be estimated rapidly, thus necessitating a rapid test to distinguish between satisfactory and unsatisfactory raw material on the basis of bacterial load.

Several workers have used dye reduction test for estimating bacterial counts in seafoods with equivocal results. Uno and Tokunage (1954) reported that the resazurin test was useful for herring, but not for mackerel. Novak et al (1956) successfully employed the methylene blue reduction test for approximation of bacterial counts in shrimps and oysters, while Cavallone (1959) found this method unreliable. Kummerlin (1982) observed that resazurin reduction time and viable bacterial count correlated well in deep-frozen shrimp. Hence, the present study was conducted to investigate the usefulness of methylene blue and resazurin reduction tests in assessing the bacterial load of different varieties of fish and finding out the suitability of employing them as rapid tests in the seafood industry for selecting satisfactory raw material.

The fish varieties used in the study were warm water marine fish viz., lesser sardine, pink perch and white prawn. Fresh raw material was brought from Tuticorin fish landing centre to the laboratory, where it was maintained in iced condition (0 to 2°C), till it became sensorily objectionable. Upon receipt, the fish samples were analysed immediately and then at intervals to obtain specimens at different stages of spoilage. Fish muscle containing skin from dorsal region was used for the analysis. Methylene blue (0.0025%) and resazurin (0.005%) solutions were prepared in sterile distilled water for use in the experiment.

The dye reduction test followed in the present study was based on the methods described by Obanu (1986) and Rao and Murthy (1986). One gram of fish muscle was taken in a test tube containing 9 ml of 0.1% sterile peptone water. Dye solution (1 ml) was then added and the tube stoppered and mixed by inverting several times. The tubes were finally incubated at 37°C and checked at regular intervals for change of colour. The contents of the tubes were initially blue for both the methylene blue and resazurin tests. The disappearance of blue colour and appearance of pink colour were taken as the end points for methylene blue and resazurin tests, respectively.

Fish muscle (10 g) was taken aseptically and homogenised in 90 ml of 0.85% physiological saline. Dilutions were made as per the requirements using 0.85% saline and plating was done on plate count agar (Speck 1976). Finally, all the plates were incubated at 37°C for 24 h.

The fish samples were also tested for their changes in odour during iced storage by the panelists. The panelists, who tested the sensory quality, belong to the faculty of fish processing technology having good experience in sensory evaluation of seafoods. Odour was scored on a scale with a range of 1-10. (9-10: excellent; 7-8: good;5-6: acceptable; 3-4: off-odour and 1-2: putrid).

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TABLE 1. CHANGES IN MICROBIAL COUNTS, ODOUR SCORES AND DYE REDUCTION TIMES OF FISHES DURING ICED STORAGE (0 TO 2°C)

Storage period, days	Sample	Aerobic mesophilic count, cfu/g	Odour scores	Dye reduction time, min	
				Resazurin	Methylene blue
	Lesser sardine	9.95×10^3	9.5	300	60
0	Pink perch	1.53×10^4	8.9	320	370
	White prawn	1.33×10^4	9.7	350	340
	Lesser sardine	2.12×10^4	9.0	250	310
1	Pink perch	2.83×18^4	7.8	260	320
	White prawn	7.55×10^4	9.0	310	300
	Lesser sardine	1.18×10^5	8.1	180	240
3	Pink perch	1.59×10^5	6.7	170	220
	White prawn	3.55×10^5	6.5	230	210
	Lesser sardine	2.04×10^5	6.9	120	190
5	Pink perch	2.80×10^5	5.4	110	170
	White prawn	2.78×10^6	5.2	160	150
	Lesser sardine	1.66×10^6	5.7	70	120
7	Pink perch	1.97×10^6	4.8	60	90
	White prawn	9.10×10^6	3.7	60	80
	Lesser sardine	4.00×10^6	3.9	60	90
9	Pink perch	6.00×10^6	3.2	40	60
	White prawn	Discontinued			

Aerobic mesophilic counts and the corresponding dye reduction times for lesser sardine, pink perch and white prawn during iced storage are presented in Table 1. In all the experiments, as storage progressed and microbial load increased, the time required to reduce the dyes decreased.It has also been observed that there exists a relation between dye reduction times, aerobic mesophilic counts and sensory odour scores. It is clear from the results that as expected, an inverse relationship exists between bacterial counts and dye reduction times in all the cases.

The present findings are in agreement with those of Reddy et al (1990a), who noted that viable bacterial counts and dye reduction times correlated inversely in many varieties of fish. Novak et al (1956) and Kummerlin (1982) reported similar relationship between baterial counts and dye reduction times for fresh and deep-frozen shrimps. Table 2 shows that the dye reduction times correlated significantly ($p<0.01$) with aerobic mesophilic counts in all the varieties. The correlation coefficients are ≥ 0.969, showing the high sensitivity of both dyes to microbial load. In this regard, both dyes were comparable. However, reduction times were generally lower for resazurin than for methylene blue at the same bacterial load (Table 1), even though these dyes are known to be non-inhibitory to microbial activity in the concentrations normally used (Saffle et al. 1961; Walker et al. 1959; Wells 1959). This indicates that resazurin dye is marginally better in determining the bacterial load of fishes. A significant inverse correlation was observed between viable bacterial counts and resazurin reduction times for raw shrimp (Reddy et al. 1990b). The results show that the dye reduction test may be used as a screening test in rapidly determining the bacterial load of raw material before they are used for further processing. The findings also suggest that when the resazurin reduction time reaches around 60 min and the odour scores are about 4.0, then the fish is not suitable for processing.

TABLE 2. CORRELATION OF DYE REDUCTION TIME (DRT) WITH AEROBIC MESOPHILIC COUNTS (AMC)

Fish variety	Dye used	Correlation coefficient of DRT Vs AMC
Lesser sardine	Resazurin	-0.975*
Lesser sardine	Merthylene blue	-0.993*
Pink perch	Resazurin	-0.969*
Pink perch	Methylene blue	-0.988*
White prawn	Resazurin	-0.983*
White prawn	Methylene blue	-0.991*

* Highly significant ($p<0.01$)

The authors thank Dr. M. Devaraj, Dean and Th. P. Jeyachandran, Head of the Department, for having provided the required facilities to carry out the study. This work was supported by the Tamil Nadu Veterinary and Animal Sciences University, Madras.

References

Cavallone E (1959) Reazioni cromatiche per stabilire la freschezza del pesce IV. Atti Soc Ital Sci Vet 12:480-481

Kummerlin R (1982) Resazurin test for microbial control of deep frozen shrimp. J Food Technol 17:513-515

Novak JH, Fieger EA, Bailey ME (1956) Rapid procedures for approximation of bacterial counts in shrimp and oyster. J Food Technol 10:66-67

Obanu ZK (1986) Evaluation of dye reduction as a quality index for raw meat under tropical conditions. J Food Sci Technol 23:46-48

Rao DN, Murthy VS (1986) Rapid dye reduction tests for the determination of microbial quality of meat. J Food Technol 21:151-157

Reddy YM, Indrani Karunasagar, Karunasagar I (1990a) Resazurin test for estimating bacteriological quality of fishes. Fisheries Research 9:75-79

Reddy YM, Iddya Karunasagar, Indrani Karunasagar, Udupa KS (1990b) The resazurin test for estimating bacteriological quality of shrimps. Asian Fisheries Sci 3:263-267

Saffle RL, May KN, Hamid HA, Irby JD (1961) Comparing three rapid methods of detecting spoilage in meat. Food Technol 15:465-467

Speck ML (1976) Compendium of methods for microbiological examination of foods. American Public Health Association Washington, DC, pp 107-120

Uno T, Tokunaga T (1954) Studies on freshness determination of fish meat by resazurin test. Bull Hokkaido Reg Fish Lab 11:78-81

Walker HW, Coffin WJ, Ayres JC (1959) Å resazurin reduction test for determination of microbiological quality of processed poultry. Food Technol 13:578-581

Wells FE (1959) Resazurin reduction tests for shelf-life estimations of poultry meats. Food Technol 13:584-586

Received 17 April 1995; revised 11 January 1997; accepted 17 February 1997

BAKED GOOD FRESHNESS, TECHNOLOGY, EVALUATION AND INHIBITION OF STALING (Food Science and Technology Series/75) - Edited by Ronald E. Hebeda and Henry F. Zobel, Published by Marcel Dekker, Inc., 270 Madison Avenue, New York, 10016, USA, 1996, pp 304, Price US $135.00/-

Quality of bakery products depends mainly on the quality and level of ingredients used, processing methods employed as well as the storage period. It is well known that storage of bakery products, particularly those belonging to intermediate moisture foods, staling is a common phenomenon. Staling makes the product brittle and hard, rendering it unacceptable, resulting in huge loss to the bakery industry. Therefore, there is a need to delay onset of staling in bakery products to enable better keeping quality and distribution.

The book entitled "Baked Goods Freshness" edited by Ronald E. Hebeda and Henry F. Zobel is an excellent compilation of various subjects related to technology, evaluation, mechanism and inhibition of staling. The book consists of eleven sections authored by eminent scientists in their fields. The book presents complete and latest information on each subject.

The first chapter on "The staling mechanism" covers different topics titled, role of starch, amylopectin and amylose, role of ordered and disordered structures in relation to staling as well as the effect of temperature and moisture on staling. The authors have discussed exhaustively the role of non-starch flour constituents like protein, moisture, pentosans, lipids and enzymes on staling of bakery products. They have also discussed the technological control of bread crumb staling.

The second chapter on 'Surfactants' covers a wide range of topics such as the role of surfactants and their mechanism of action in delaying the staling, the interaction of ingredients and surfactants. The current use of surfactants in inhibiting the staling is included as concluding part, wherein common surfactants and polar lipids as emerging natural dough conditioners are also covered.

The use of enzymes in baking industry is known from ancient times. In the third chapter, the role and use of enzymes especially amylolytic enzymes has been briefly but precisely brought out. Though staling of bread/baked products is widely related to amylose and amylopectin architecture, remedy for it by way of using amylolytic enzymes, their action pattern/mechanism of antistaling is described fairly in detail.

The fourth chapter deals with non-amylolytic enzymes and their role in staling. The enzymes covered are non-starch polysaccharide degrading enzymes and lipid modifying enzymes. At the end, the authors have thrown light on the effect of flour contituents and enzymes in staling mechanism.

The fifth chapter depicts various methods to measure staling. Firmness measurement using different instruments are discussed at length. Dynamic rheometry and thermal methods are also highlighted.

In the next chapter, sensory methods to measure staling are discussed. The authors have described sensory attributes for different bakery products. Relationship of quantitative sensory textural measurements to instrumental measurements of mechanical properties is also discussed.

In the seventh chapter on 'Preservatives', various factors affecting microbial growth such as available nutrients, storage temperature, level of acidity, water activity and sanitation practices are described. Several chemical preservatives and their effectiveness against yeast, moulds and bacteria are discussed. At the end, a brief mention is made about natural preservatives.

Eighth chapter deals with 'Modified atmospheric packaging of bakery products'. Spoilage problems including physical, chemical and microbial are described. Shelf life extension using modified atmosphere packaging is discussed. It includes growth of MAP technology, gas packaging, factors influencing the antimicrobial effect of carbon dioxide. Advantages and disadvantages to extend shelf life of bakery products is also discussed.

In the ninth chapter on 'A Baker's perspective of baked goods', the requirement of baker with respect to softness of baked goods, interrelationship of softness with freshness, moistness, and density are discussed. Use of softeness enhancing ingredients such as fats and oils, fat-based softners (emulsifiers), protein-reactive ingredients (SSL, CSL and DATEM), antistaling enzymes, emulsifying ingredients, gums and fibres, oxidising and reducing agents and structural ingredients to retain the freshness of baked goods is discussed.

In the next chapter on 'Consumer's perception', a brief review of staling mechanism, instrumental and sensory measurements of staling, analytical versus tactile analysis, consumer tactile testing is

discussed. At the end, improvement of shelf life and their effect on market and consumer satisfaction is illustrated.

In the last chapter on 'Labelling and regulatory' requirements', labelling laws and regulations, national uniformity, labelling claims for freshness, FDA's position, definition and rule on 'fresh' labelling, labelling preservatives, statutory, regulatory requirements, chemical and "no preservative" claim are described. Finally, use of enzymes for preservation of freshness is briefed.

This practical reference book offers in a single volume, covering the whole gamut of staling process that occurs upon ageing in baked goods in detail with over 800 bibliographic citations, figures and tables. This comprehensive book is a source of reference to cereal chemists, biochemists and other research workers in the field of Food Science and Technology. It is also a useful book for teachers and students of Food Science and Technology.

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APPLIED PLANT BIOTECHNOLOGY, Written by Rev. Fr. Dr. S. Ignacimuthu, S.J. Published by Tata McGraw Hill Publishing Co Ltd. New Delhi, pp 314, Price Rs 240/-

The 1980's witnessed rapid advances in industrial biology, particularly in plant science and it has emerged as Applied Plant Biotechnology. The advent of plant biotechnology opened up new opportunities for co-operative improvements in agriculture, forestry, energy, food, pharmaceutical, cosmetics and service industries. The key areas of plant biotechnology applications are genetic engineering, plant tissue culture, cryopreservation, bioreactor, fermentation and treatment technologies. Gene isolation, transformation, regeneration, molecular maps and DNA probes based on RELP/RAPD, transgenic plants and seeds are the frontier and emerging areas of research to promote biomass growth and agricultural productivity. This plant biotechnology is contributing to the welfare of humanity in many different ways. Thus, the subject has become an important and integral part of any country's developmental programme and plant biotechnology as a course is introduced in all life science subjects. The book on Applied Plant Biotechnology by Rev. Fr. Dr.S. Ignacimuthu, S.J. is timely and helpful to the students as well as researchers and the teachers in industries, universities and colleges. It provides some relevant and essential information on applied plant biotechnology as used in agriculture and industry.

The book contains 4 chapters. The first chapter covers the subject on 'Crop improvement and plant biotechnology'. It deals with isolation and characterization of plant genes, development of transgenic plants and their applications, with examples on attempts to modify chemical composition to improve quality of grains, seeds and nutritional traits. Implication of plant tissue culture for crop improvement has been discussed extensively. The major chapters include exhaustive list of selectable markers and reporter genes used in plant genetic transformation, examples of cloned plant genes, plant species in which transgenic plants have been obtained, linked markers and genes imparting resistance to pathogens, properties of seed storage proteins from some major crop species, aspects and uses of plant tissue culture, plant regeneration from embryogenic callus of cereals.

The second chapter describes present status and improvement aspects with examples on agriculture and plant biotechnology, outling the work on micropropagation application and advantages, plant disease elimination techniques, germplasm sources and cryopreservation techniques, protocol and guide lines, biological nitrogen fixation, gene-regulation and expression and biofertilizers. The presentation helps both students and researchers for a concise understanding of the subject.

Industry and plant biotechnology areas are projected in chapter 3. Biosynthesis of plant compounds, production of secondary metabolites, bioinsecticides for storage of foodgrains, large scale cultivation of food value metabolites have been described lucidly. In addition, major groups of compounds with commercial importance derived from plants and their associated industries, important drugs from plant source, accumulation of secondary metabolites in cell and tissue culture, synthesis of biochemicals using immobilized cells of some plant species, elicitor-induced product accumulation, biotransformation by plant cell culture, important food additives from plant cell culture, trade in floriculture are covered extensively and appropriately.

Biomass and plant biotechnology development are explained in the fourth chapter. Plant biomass, sources of biomass such as forest biomass availability, crop residues, aquatic biomass, water as a source of energy, composition of plant biomass, biomass conversion, ethyl alcohol fermentation,

methanol using wastes, methane from sewage, farm and industrial wastes, biogas technology in India, energy sources, pulp and paper, oxychemicals, single cell proteins and mycoproteins, mushrooms and control of pathogens and pests are vividly covered. Major biomass resources in India, use of wood distillation products, examples of renewable sources of biomaterials, chemical composition of lignocellulosic material, alcohol production from some important crops, and major sources of naturally produced methane are included in the text, which is laudable. Biomass as the source of energy, oxychemicals from biomass, microbial production of chemicals, single cell protein and mycoprotein produced on the selected substrates, distribution of edible mushrooms in India, compost formulations used are dealt in succinct form, which is a very valuable information to the reader.

In each chapter, the author has thoughtfully included the study questions to benefit the reader, particularly the students. Selected references are also included so as to seek further information on the important aspects covered in each chapter. Glossary provided at the end is highly useful to the students. The text is profusely illustrated with figures and tables. Dr. M.S. Swaminathan, who has written the foreward is of the opinion that the book is a timely contribution and expects that the book will be read widely.

The text book has made a sincere attempt to provide readers with information to cope up with the fast-growing area of plant biotechnology. Almost all aspects of plant biotechnology are well summarized and I am sure the book will serve as a basic text for any core course on biotechnology. It also stimulates interest among entrepreneurs, as it enlightens the achievements that are possible in the years to come.

The book on the whole is well written. The author and publisher have taken great care to offer to the reader one more text book to the never ending demand for specialised text books in the field of biotechnology.

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Published by the Secretary AFST (India) CFTRI, Mysore-570 013 and Printed at M/s. Jwalamukhi Job Press, 44/1, K.R. Road, Bangalore-560 004, India.