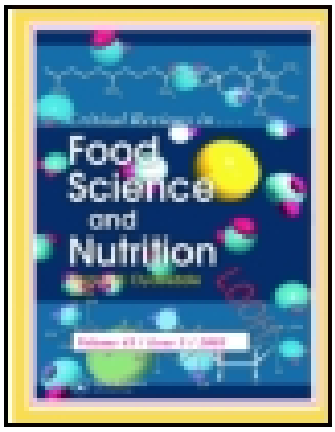

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Ways and means for the infusion of bioactive constituents in solid foods

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Abstract

The development and consumption of functional food, or foods that promote health not merely basic nutrition, is on rise. In recent years, industrial and consumer interests have focused on developing foods supplemented with bioactive constituents that provide greater physiological benefits. The direct addition of these components to liquid or fabricated solid foods has led to a wide range of new products appearing on the market. Osmotic dehydration, an operation in which food stuff is soaked in solution of low water activity, has been reported as a suitable technology for formulating new products because of the twofold effect that it has on food where it partially removes water and impregnates the food pieces (solid food matrix) with solutes from the osmotic solution. The article focuses on the impregnation of bioactive constituents having added advantage to human health such as antioxidants, minerals, vitamins and probiotics. The infusion of enzymes and aroma also has been discussed. Application of ultrasound, vacuum, high pressure and/or atmospheric impregnation techniques appears to be the feasible technologies for impregnation of solid food matrix for the incorporation of bioactive ingredients.

Key words: Bioactive compounds, impregnation, vacuum impregnation, osmotic dehydration, ultrasound.

1. Introduction

In recent years, interest has grown in development of new functional foods that have health promoting and/or disease-preventing properties beyond the basic function of supplying nutrients (Rozek et al., 2010). Today there is unprecedented interest by consumer, public health organizations, and the medical community to improve health and wellness through dietary means. A food can be regarded as functional if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either improved stage of health and well being and/or reduction of the risk of diseases (Diplock et al., 1999; Alzamora, 2005).

Increased consumer interest in the health benefit of foods has led to significant development of nutraceutical and functional foods (Zhao and Xie, 2004). In 2013, the estimated global market of functional food industry is expected to reach 176.7 billion USD with a compound annual growth rate of 7.4% (Roberts, 2009).

Fruits and vegetables are rich in naturally occurring bioactive compounds, which are the important components of a healthy diet. Natural materials are an emerging field in food science because of their increasing popularity with consumers concerned about health. American Nutraceutical Association has defined nutraceutical as a substance that is a food or isolated or purified from foods, which provides medical or health benefits including the prevention and treatment of diseases. These products may include isolated nutrients, dietary supplements and specific diets to genetically engineered designer foods, herbal products and processed foods such as cereals, soups and beverages. Antioxidants, natural colorants (e.g. lycopene, cartenoids), minerals, probiotic bacteria or vitamins are the several examples of bioactive compounds, which provide added health benefits. For example, a colorant besides being functional health ingredient also enhances marketability, improves sensory attributes and influences consumer behaviour of the product.

Mixing the bioactive active compounds with foods can be a useful procedure for formulating liquid foods (such as milk or yoghurts enriched with calcium, iron, vitamins) or restructured foods (such as breakfast cereals, cakes or bread). However, this technique is not very appropriate for production of functional food from solid foods, which maintain their natural matrix or cellular structure (Fito et al., 2001). The knowledge of food matrix composition, structure and properties is essential to control or enhance the infusion and to study the changes resulting in the sensory, functional and nutritional quality.

Osmotic treatment (osmotic dehydration or dewatering impregnation soaking) seems to be a feasible technology for the development of fruit and vegetable matrices to which functional ingredients can be successfully added to provide novel functional product and new commercial opportunities (Alzamora et al., 2005). Osmotic treatment had many fold advantages, which include food formulation by reducing the water activity and supplementing the foods with compounds that modify its functional, structural and nutritional properties (Spiess and Behsnilian, 1998; Torreggiani and Bertolo, 2001; Fito et al., 2001; Alzamora et al., 2005). During osmotic dehydration, several flows take place such as water removal from food material to hypertonic solution and, simultaneously, in counter current solute uptake. Additionally, soluble compounds of the foodstuff can accompany the water flow. Several mechanisms such as osmosis, diffusion and hydrodynamic mechanisms take part in the mass transfer phenomena (Rastogi et al., 2000; 2002). The biologically active compounds are transferred from surrounding solution to the food by a process of diffusion in which naturally occurring cell membrane structure functions as a semi-permeable membrane. The intercellular spaces present in the natural food matrix decide the extent of infusion of biologically active compounds. The kinetics of infusion, viability of infusate, interaction

between the food components, cellular structure and mechanical properties are the few major concerns regarding the infusion of the biologically active compounds in solid foods. Gonzalez-Paramas et al. (2004) also have regarded osmotic treatment as a method that changes the food formulation by reducing the water content as well as supplementing the food with compounds that modify the functional and nutritional properties.

Mass transfer during osmotic treatment occurs through the semi-permeable cell membrane that offers the dominant resistance to mass transfer in biological materials. The state of the cell membrane can change from partial to total permeability, leading to significant changes in tissue architecture (Rastogi et al., 2002). The major limitation of osmotic dehydration process is the cost of osmotic solution that necessitates a proper means of its recycling. During osmotic dehydration the solution gets diluted and gains certain flavour, and colour, the extent of which depends upon the type of the food. Care should be taken to minimise these so that product quality is not affected by recycling the osmotic solution. The osmotic solution needs to be concentrated by some means (evaporation and/or addition of solute) so that it can be recycled (Rastogi et al. 2002).

A number of techniques have been proposed to enhance the inherently low rate of osmotically induced mass transfer include partial vacuum (Rastogi and Raghavarao, 1996; Fito et al., 2001), high pressure (Rastogi et al., 2010) or ultrasound (Rastogi et al., 2011).

Application of partial vacuum to a porous tissue surrounded by a solution containing bioactive compounds results in extraction of air contained in the intercellular spaces and then on restoration of pressure, the impregnation solution penetrates intercellular spaces by hydronamic mechanism consisting of capillary action and pressure gradient. This substitution of air with impregnating solution allows direct formulation of a food by modification of solid matrix without exposing it to eventual stress due to long exposure to highly concentrated solution at atmospheric pressure (Alzamora et al., 2005).

The application of high hydrostatic pressure to fruits and vegetables affects cell wall structure, leaving the cell more permeable, which may be in turn beneficially used to enhance the uptake of biologically active substances from the surrounding solution. Application of high pressure has been reported to accelerate the diffusion of components into the food. Pressure causes structural transformations in the food, which may alter the diffusion coefficients. Rastogi and Niranjan (1998) have demonstrated that the application of pressure pretreatment resulted in increased rate of water and solute diffusion during osmotic dehydration of pineapples. They indicated that in case of pineapple, the high pressure treatment resulted in breakdown of cell walls (increased size of cells due to reduction in the intercellular material when samples were subjected to a high pressure and decompressed) and leaving cell more porous resulting in softening of the tissue. Application of high pressure up to a certain extent was demonstrated to enhance the diffusion of sucrose in potato cylinders (Sopanangkul et al., 2002) and water uptake in glutinous rice (Ahromrit et al., 2006). Villacís et al. (2008) also demonstrated increased diffusion of NaCl in turkey breasts meat due to the application of high pressure.

High intensity electrical field pulse treatment has been reported to increase the permeability of plant cells (Knorr et al., 1994; Knorr and Angersbach, 1998). High intensity electrical field pulse pretreatment was found to accelerate effective diffusion coefficients of water and solute during osmotic dehydration facilitating the transport (Rastogi et al., 1999). Later, Phoon et al (2008) demonstrated that a disaccharide trehalose can be impregnated in the spinach leaves using pulsed electric field in combination with vacuum application thus improving the freezing tolerance of spinach leaves.

The use of ultrasound in combination with osmotic treatment results in higher rate of water loss and solute gain at a lower solution temperature, while preserving the natural flavor, color and heat-sensitive nutritive components. The application of ultrasound results in

acoustical cavitations leading to the production of minute vapour-filled bubbles that collapse rapidly, resulting in complete and accelerated degassing of immersed solid. Ultrasound wave travelling through a solid medium can also generate a series of alternative compressions and expansions, in a similar way to a sponge when it is squeezed and released repeatedly (sponge effect) (Stojanovic and Silva, 2007). The higher surface tension force caused by this mechanism maintains the moisture inside the capillaries of the material creating microscopic channels (increased cell permeability or lower resistance), which may make the moisture removal easier (Carcel et al., 2007; Deng and Zhao, 2008). Also, the oscillatory motion of a sound wave causes acoustic streaming, which leads to the enhancement of mass transfer (Fernandes et al., 2008; Rastogi, 2011).

A good number of opportunities exists and have been reported in literature regarding the infusion of bioactive compounds in solid foods using various techniques. These compounds include antioxidants, minerals, vitamins or probiotics. A few selected applications have been discussed in the following sections. A list of the important findings in this regards have been presented in Table 1.

2. Infusion of antioxidants

2.1 Infusion of grape phenolic

Grape is one of the world's largest fruit crops, and the press residues resulting from winemaking industry are rich in phenolics, which is generated in huge amounts as by-product. These extracts are usually a complex mixture of phenolic compounds such as flavonoids (catechin, epicatechin, procyanidins and anthocyanins), phenolic acids (gallic acid, ellagic acid), stilbenes (reservatol and piceids) and other phenolics. Ozkan et al. (2004) reported the antioxidant and antimicrobial properties of grape pomace extracts.

Rozek et al. (2007a) demonstrated that osmotic dehydration can be used as an operation for supplementing a solid foodstuff (model food gel) with grape phenolics from a concentrated red grape must to increase its antioxidant properties. The water loss (ΔM^{w}), soluble solids (ΔM^{SS}), total phenolics (ΔM^{TPH}) and fraction $\Delta M^{w}/\Delta M^{TPH}$ were found to increase increased with time and also with the soluble solids concentration of the osmotic solution (**Fig. 1**). Osmo-dehydrated food with a higher total phenolic content (up to 7000 mg GAE/kg) was produced in shorter processing times (1 or 2 h) of osmotic dehydration. Rozek et al. (2007b) indicated that the molecular weight of the phenolics had a significant impact on their infusion rate. The results showed that the penetration of phenolics of molecular weight above 600 g/mol made a poor contribution to total phenolic impregnation. The osmotic solute concentration of 40 and 50°Brix resulted in increase in diffusion coefficient of total phenolics, whereas, the diffusion coefficient of individual phenolics such as hydroxycinnamates (caftaric and coutaric acid) and hydroxycinnamic acids (caffeic, coumaric, and ferulic) did not change significantly. However, during osmotic dehydration with 60°Brix, the diffusion coefficients of individual as well as total phenolics were found to decrease significantly.

Rozek et al. (2008) used mixtures of two osmo-active solutes (NaCl and sucrose) and commercial grape seed extract in the osmotic solution to formulate a model food enriched with grape phenolics by osmotic treatment. Phenolic infusion into model food varied significantly with the ratio of sucrose to NaCl mole fraction in osmotic solution. At a certain concentration of sucrose (0.95 m) and sodium chloride (1.46 m), the flavan-3-ol monomers and dimmers were found in concentrations of 1334 and 486 mg/kg, respectively.

Rozek et al. (2009) showed that the nature of osmoactive solutes significantly affects the mass transfer rate of grape phenolics in a solid model food. Of all the osmo active solutes sodium chloride resulted in the highest total phenolics infusion rate (**Fig. 2**). The highest

infusion was due to lowest molecular weight of osmotic active agent. The total phenolic content of the model food was similar to or higher than that of the fruits and vegetables rich in phenolic content.

Rozek et al (2010a) studied the infusion of grape phenolic compounds into a model food (agar gel), fruits (apple and banana) and vegetable (potato) plant tissues. The stability of the grape phenolics after a post-treatment such as convective air drying was evaluated. It was concluded that the extent of grape phenolic impregnation was controlled by food structure and the kind of osmo-active solute. The total increase in phenolics was found to be linearly related with the solute gain during the osmotic treatment (**Fig. 3**). Further, Rozek et al (2010b) compared the effect of sucrose in the osmotic solution on the infusion of total phenolics. Osmotic solution containing the same phenolic content but with no sucrose in osmotic solution resulted in much higher infusion of total phenolic content in the model food system.

2.2 Infusion of curcuminoids

Turmeric (*Curcuma longa* L) is used as a food coloring agent and has been found to be a rich source of phenolic compounds namely cucuminoids with the principle ingredient being curcumin and other two analogues are demethoxycurcumin and bisdemethoxycurcumin (Govindarajan, 1980). Curcumin is regarded as a body cleanser in Ayurvedic system of medicine and it has a capacity to heal many diseases. It has been shown to exhibit pharmacological properties such as anti-inflammatory, antimicrobial, antioxidant, antiparastic, antimutagenic and anticancer activity (Goel *et al.*, 2008,). The use of curcuminoids in fruit bread and ice-creams were found to be highly acceptable (Sowbhagya et al., 2005). Maximum acceptable daily intake for curcumin is 0.1 mg/kg body weight (FAO, 2000).

Bellary et al. (2011) explored osmotic treatment as a method to infuse curcuminoids in coconut slices. The rate of mass transfer of moisture, solid and curcuminoids with or without application of ultrasound were studied over a range of concentration of osmotic solutions (0 to 50%). Increase in the concentration of osmotic solution beyond 25% resulted in reversal in the direction of moisture and solid mass transfer (**Fig. 4a and 4b**). The increasing concentration of osmotic solutions (0, 12.5, 25 and 50%) used for the incorporation of curcuminoids indicated that the highest incorporation of curcuminoids could be achieved when the concentration of surrounding solution was minimum i.e. 0%. The curcuminoids concentrations continue to increase with immersion time, but the rate and extent of curcuminoids impregnation was less than that of the situation when water was used as a surrounding solution (**Fig. 4c**). Ultrasound treatment resulted in higher moisture, solid and curcuminoids mass transfer due to the breaking of cell structure as revealed by microstructure examination (**Fig. 5**). HPLC analysis revealed that all the curcumionds (curcumin, demethoxycurcumin and bisdemethoxycurcumin) were infused into the coconut matrix. The study concluded that osmotic treatment can be used as a feasible technology for impregnation of functional ingredients into foods without altering its matrix.

Bellary and Rastogi (2012) studied the impregnation of curcuminoids into coconut slices using the surrounding hypotonic or hypertonic solutions. The rate of curcuminoids infusion into coconut slices was directly related to solution concentration as well as the osmotic pressure inside the solid food matrix due to presence of endogenous soluble solutes. At the critical concentration of surrounding solution, osmotic pressure of the surrounding solution becomes equal to the osmotic pressure inside the cells and no transport of moisture or solute occurs. Below critical concentration of surrounding solution, the diffusion of moisture into the coconut slices and diffusion of solids from coconut slices to surrounding solution took place. The direction of the mass transfer reversed when the concentration of

surrounding solution was above critical concentration (**Fig. 6**). The use of low concentration of sodium chloride (2.5%) resulted in increase in infusion of curcuminoids as compared to that of a situation when water as taken as surrounding solution.

2.3 Infusion of quercitin

Apple peels contain quercetin (flavonoid) more than apple parenchyma in the form of glycosidic bonded sugars such as quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-xyloside, quercetin-3-arabinoside, quercetin-3-rhamnoside, and quercetin. These act as antioxidant, which are the important determinants in protecting against many diseases. Schulze at al. (2012) studied the infusion of apple peel quercetin into apple cubes by involving vacuum impregnation. The quercetin infusion in apple slices was depended on level of vacuum applied, soluble solid content and viscosity of osmotic solution. A slight but significant increase in the enrichment of the apple tissue was found when lowering the vacuum pressure from 800 mbar to 600 mbar. However, a 2.5-fold increase was found when the vacuum level changed from 200 mbar to 100 mbar (**Fig. 7a**). When decreasing soluble solid content from 11°Brix to 0.3°Brix, a marked increase in the flavonoid content was found (**Fig. 7b**). The use of hypotonic external fluids increased the penetration of the quercetin solution, due to the osmotic pressure. The hypotonic liquid passed the cell plasmalemma into the hypertonic cytoplast. The mass transfer into the cells enhances the vacuum impregnation and the cellular turgor (Pitt, 1992). Therefore, the highest fortification of the sum of quercetin derivatives was achieved. The quercetin enrichment after impregnation at 100 mbar of the pectin-enriched apple juice solution was 50% lower compared to the corresponding vacuum impregnation of the pectin water solution (**Fig. 7c**). This phenomenon was attributed to the higher osmotic pressure and viscosity of the external solution of apple juice containing pectin.

3. Infusion of minerals

Health benefits are one of the specific issues that will greatly influence the food industry in the next few years. In this sense, the functional food industry has become one of the most promising sectors of the food industry in the last few years (Mazza, 1998). Fortified foods with minerals can deliver potential benefits in terms of health maintenance and disease prevention. Some of the important minerals beneficial for health, namely calcium and zinc, are present in low concentrations in most of the fruits and vegetables, which can be enhanced by adopting various impregnation techniques.

One of the most interesting fortifiers in the functional food development is calcium. Calcium content of diet is critical in most stages of life. This mineral is chiefly found in dairy products, but these are sometimes the cause of digestive troubles. Calcium is, in turn, involved in superior plant architecture, forming bonds between pectins and other cellular wall components (Brett and Waldron, 1990).

Fito et al. (2001) modelled the vacuum impregnation of calcium in oranges, eggplants and other fruits and vegetables using different calcium sources. The mass of different fruits that had to be consumed to get 20% of RDI was plotted against the concentration of calcium lactate in impregnation solution. The curves for each fruit had been obtained with in the solubility range of calcium lactate. The dashed line indicates the cases where the value of fruit range range below 200g (**Fig. 8**). Microstructure of vacuum impregnated of both orange peel albedo and eggplant indicated wide intercellular spaces responsible for the high porosity values. The presence of this solution filling the sample voids is reflected trough the continuous dentric aspect of the sample occluded in this phase (**Fig. 9).** Rico et al. (2007) compared the effect of heat-shock, calcium lactate and chlorine treatment on the textural properties of carrot. Calcium lactate treatment produced reduction in the water activity in sliced carrots and higher firmness as compared to chlorine treatment. In addition, the

treatment increased polymethylesterase activity significantly in comparison to other treatments, which resulted in higher firmness as confirmed by sensory analysis (**Fig. 10**). Cryo-SEM analyses showed that combined heat-shock and calcium lactate treatment were more effective in maintaining the turgor of cortex tissue cells.

Grass et al. (2003) demonstrated that the calcium fortification of vegetables such as eggplant, oyster mushroom and carrot was obtained by vacuum impregnation. The calcium ions interacted with plant tissues and resulted in the modification of its mechanical properties as well as extent of impregnation. The level of impregnation and deformation indicated that infusion of calcium slightly influenced the impregnation behaviour of all the vegetables. However, mechanical behaviour of eggplant and carrot were markedly affected by calcium, and no significant effects were observed in oyster mushroom due to absence of pectin in their cell architecture. Energy dispersive X-ray microanalysis showed that calcium impregnation occurs in the intercellular spaces of eggplant and oyster mushroom and to a less extent in xylem of carrot. The eggplant and oyster mushroom reached the greatest final impregnation level because of their great effective intercellular porosity as compared to carrot. The cells in the carrot external parenchyma are very densely packed and no intercellular volume was available for vacuum impregnation (**Fig. 11**).

Alzamora et al. (2001) and Anino et al. (2002; 2006) studied the capacity of calcium impregnation of parenchymatous apple tissue by different impregnation techniques (atmospheric impregnation or vacuum impregnation) and the effect of these treatments on mechanical properties. Xie and Zhao (2003) evaluated the use of vacuum impregnation for developing nutritionally fortified fresh cut apples. The results indicated that 15-20% of the daily reference intake of calcium and above 40% of the daily reference intake of zinc could be obtained in 200 g fresh cut apples. The vacuum impregnation had little effects on the

physicochemical properties of apples. The presence of zinc significantly improved color stability and calcium enhanced the firmness of the apples.

Anino et al. (2006) analyzed the ability of apple matrix for calcium incorporation by vacuum as well as atmospheric pressure impregnation techniques and determined the effect of these treatments on material compression behaviour. The greatest calcium content (3100 ppm) was reached after 22 h atmospheric impregnation process, while the amount of calcium incorporated during vacuum treatment was similar to the one obtained after 10 h. Calcium impregnated tissues exhibited different response to compression as compared to raw fruit, showing a decrease in the values of rupture force (F_{rup}) and modulus of deformability (E_D). Impregnation with calcium under atmospheric conditions up to 2 h resulted in increase in rupture force and no significant change in modulus of deformability (E_D). However, further increase in impregnation time resulted in decrease in these parameters (**Fig. 12a and 12b**). Modulus of deformability (E_D) for samples treated under vacuum was significantly lower than that obtained for the control. When Ca^{2+} was added, the loss of tissue rigidity was again evident as compared to the treatment under atmospheric pressure (**Fig. 12c and 12d**).

Barrera et al. (2004) studied the effect of calcium and iron ions incorporation in the structural matrix of apple slices on its behaviour during the osmotic dehydration process. Low concentrations of minerals employed do not affect net changes of mass, water and solutes of impregnated samples. The incorporation of calcium diminished effective diffusivity values because it promoted bonds formation in middle lamellae and cell walls, which resulted in increase in the elastic behaviour, stiffness and fragility of the cellular network. This phenomenon was not observed in the case of samples impregnated with iron ion. Barrera et al. (2009) studied the effect on the mass transfer kinetics during osmotic dehydration of the quantity on calcium incorporated into apple slices. The leaching of calcium content during the process was reduced by adding certain amounts of calcium to the

osmotic solution. Despite the loss of part of the calcium incorporated to apple slices by means of vacuum impregnation, osmotic dehydration can be considered as a useful tool to increase the stability of the product without seriously reducing its nutritional value.

Escalada Pla et al. (2009) demonstrated that pumpkin processed through hurdle technology involving decrease in water activity and use of antimicrobial as well as pH depressor could be impregnated with iron to provide a proportion of recommended daily intake of iron. The presence of iron in the product did not produce significant changes in microbiological performance, organoleptical behaviour during storage, firmness, colour degradation and sensorial quality. However, a slight reduction in pH and a_w was noticed. All the iron present was found to be biologically available according to results obtained from *in vitro* digestion with pepsin and pancreatin/biliary salts.

Luna-Guzman et al. (2000) indicated that calcium lactate treatment is a potential alternative to calcium chloride for shelf life extension of fresh-cut cantaloupe, since it provided similar or better tissue firming without providing undesirable bitterness. A calcium lactate or calcium chloride treatments at either 25 or 60°C resulted in significantly firmer samples than the sample treated with water alone at the corresponding temperatures during storage. However, maintenance of firmness tended to be higher in calcium lactate in comparison to calcium chloride treated samples throughout storage (**Fig. 13**)

Tapia et al. (2003) studied the impregnation of melon with calcium under vacuum. The maximum level achieved (319 ppm) represented only 2.2–2.9% of the adequate intake per portions of 100 g of melon. Sensory evaluation showed that enriched samples were having high acceptability than that of fresh samples.

Monsalve-Gonzalez et al. (1993) studied the incorporation of calcium to prevent softening of fruit cylinders during osmotic adjustment of water activity by immersion in sugar solutions. The decrease in the compression force was observed during the first 2 h of

osmotic treatment along with the water loss and sugar gain. This softening could be minimized by addition of calcium chloride (1000–2000 ppm) to the impregnating solution. Gonzalez-Fesler et al. (2008) studied the effect of blanching and calcium impregnation at vacuum impregnation and atmospheric pressure on the rate of water movement during drying of apples. Effective diffusion coefficient was strongly affected by preheating of apple as well as by tissue contraction. With the exception of non-blanched tissues subjected to vacuum impregnation or vacuum impregnation followed by atmospheric pressure, calcium uptake during impregnation step appeared to modify the matrix resistance to water flux thus improving the wall structure of blanched tissues.

Ortiz et al. (2003) evaluated the viability of highly porous matrix of mushroom for the incorporation of calcium by vacuum impregnation. A significant tissue softening was observed in the sample after vacuum impregnation of calcium. The deteriorative effect of vacuum impregnation on textural characteristics was slightly reduced by calcium addition. The softening of the sample was due to cytoplasmolysis and disruption of cellular membranes, loss of turgor and collapse of cells.

Moraga et al. (2009) evaluated the effect of calcium lactate on osmotic dehydration kinetics, respiration rate, mechanical properties and shelf-life of fresh, vacuum impregnated as well as pulsed vacuum osmo-dehydrated grapefruit. The shelf-life of 5 to 8 days was achieved due to osmotic dehydration. It was increased to 11 days if calcium was added to the osmotic solution. At the same time no significant effect on the mechanical properties of the sample was observed. The dehydration of samples resulted in decrease in the cellular respiration rate and, consequently, an increase in the shelf-life of the processed fruit, both of which were more significant if calcium is added to the sample. The decrease in water effective diffusivity due to the presence of calcium indicated the interaction of calcium ion with the aquaporins of the plasmalemma membrane resulting in increase in the ATP

concentration inside the cell, which justified the observed decrease in the respiration metabolism.

Rastogi et al. (2008) demonstrated that complementing calcium treatments with mild heating and application of high pressure can be used as method for the improvement in the texture of carrot during thermal as well as pressure-assisted thermal processing. The use of combined pretreatments (1.0% $CaCl_2$, 200 MPa, 60°C) resulted in increase in the hardness of thermally processed samples and pressure-assisted thermally processed sample by 9.16 times (from 14.08 to 129.07 N) and 13.22 times (from 4.36 to 57.63 N), respectively. The enhanced diffusion of calcium due to combined effect of pressure and temperature resulted in firmer plant tissue by binding to pectin carboxyl groups that are generated through the action of PME. Microstructure analysis of pressure-assisted thermally processed carrots indicated that combined pretreatment was able to maintain cell structure (**Fig. 14**). Further, Rastogi et al. (2010), optimized the conditions using a graphical optimization technique and practical optimum zone (pretreatment pressure 270–288 MPa and temperature 57.7–59.3°C) was deduced as workable optimum conditions meeting following the criteria for hardness ≥145 N, calcium content ≥2.5 g/mg, and PME activity ≥70% . Sila et al. (2004) indicated that high pressure treatment of carrots combined with $CaCl_2$ infusion improved texture during thermal processing. Moreover, carrot subjected to high pressure pretreatment alone resulted in less loss of texture when these were further processed at high temperatures.

4. Infusion of vitamins

Lin et al. (2006) demonstrated a novel approach for the incorporation of vitamin E using honey-based vacuum impregnation solution to develop high-quality fortified fresh-cut pears. Vitamin E content of pears impregnated with honey solution containing α- tocopherol-acetate, free α-tocopherol, or water-soluble α-tocopherol-acetate increased 80 to 100 times

and 65% to 80% vitamin E activities were retained during 2 weeks of storage. Microbial counts were under 2.6 log CFU/g at the end of 7 days storage and the product had significantly lower browning index, higher lightness and consumer acceptance rating than control. Similarly, Park et al. (2005) impregnated Vitamin E (0.4%) in fresh-cut apples using high fructose corn syrup and hydroxypropyl methyl cellulose/ calcium caesinate as osmotic solution by applying vacuum. As a result, α-tocopherol acetate content was increased up to 21.8 mg/100 g, using high fructose corn syrup followed by 12 and 20.2 mg/100 g were achieved when using 1% hydroxypropyl methyl cellulose and 1% calcium casinate as vacuum impregnation solution, respectively. Calcium and zinc content were also increased to 146.6 and 1.7mg/100 g, when 7.5% gluconal cal and 0.04% zinc lactate were added.

Santacruz-Varquez et al. (2008) compared the enrichment of apple slices with β-carotene by osmotic dehydration and by pulsed-vacuum osmotic dehydration. The increase in concentration of osmotic solution from 30 to 50 °Brix resulted in increase in β-carotene from 1.5 to 4.1 mg/g dry solids under osmotic dehydration and 4.7 to 6.0 mg/g dry solids under pulsed vacuum osmotic dehydration, respectively (**Fig. 15**). High sucrose concentration in solution induced high levels of impregnation due to high driving forces achieved between the fruit and the hypertonic solution containing β-carotene. The pulsed-vacuum osmotic dehydration was indicated to be a potential alternative technique for the impregnation bioactive compounds.

Ascorbic acid is an essential component of most living tissues. It plays an important role in protection against oxidative stress as an antioxidant. It is an important scavenger of free radical species such as reactive oxygen species that can cause tissue damage resulting from lipid peroxidation, DNA breakage or base alterations, and may contribute to degenerative diseases, such as heart disease or cancer. The current recommended daily

allowance for ascorbic acid is suggested to be 100 mg/day for adults to achieve cellular saturation and reduce risk of heart disease, stroke and cancer, in healthy individuals.

It was shown by Hironaka et al. (2011) that the vacuum impregnation treatment increased the ascorbic acid content of whole potatoes ten times (150 mg/100 g) (**Fig. 16a**). The effects of cooking and storage time in subsets of the fortified samples were also evaluated. At 60 min of vacuum impregnation of potato had a 21 times higher ascorbic acid content than that of raw potatoes. In addition, vacuum impregnation potato beyond 30 min of vacuum time, had ascorbic acid content in excess of the FAO values (45 mg/100 g).

The ascorbic content of vacuum impregnated potatoes by steam-cooking was found to decrease by 42%. However, the vacuum impregnated cooked potatoes had 22 times higher ascorbic acid content than that of cooked sample not subjected to vacuum impregnation. In addition, vacuum impregnated-cooked potatoes had 90 mg/100 g ascorbic acid content, which was over twice the FAO value (**Fig. 16b**). The storage study showed that vacuum impregnation whole potatoes had a relatively high ascorbic acid concentration (50 mg/100 g fresh weight), even at 14 days of storage at 4 °C (**Fig. 16c**).

5. Infusion of pectin methyesterase enzyme

Pectinmethylesterase (PME) is a cell wall bound enzyme, which de-esterifies pectin. Its action liberates free carboxylic acid, which could successively interact with calcium. The activation of PME has also been reported to contribute to the firming effect of calcium (Alonso et al., 1995). Degraeve et al. (2003) demonstrated that replacement of occluded air present in the porous fruits like apples, strawberries and raspberries using classical infusion or vacuum impregnation technique by the osmotic solution containing pectin methyl esterase and calcium chloride resulted in firmness of the fruits (**Fig. 17a and 17b**). In order to optimize the concentration of PME content and holding time at 40 °C, the strawberry halves

were subjected to a solution containing 2.5% (w/w) $CaCl_2.2H_2O$ and different concentration of PME preparation (0 to 3%, w/w) for different pretreatment time (0 to 20 min). For holding times of 0 min, 10 min, and 20 min, the optimal concentration of PME preparation for highest firmness were 0%, 0.12%, 3%, and 6%, respectively (**Fig. 17c**). These results clearly indicate that a reduction of the activation period should be compensated by an increase of the concentration of PME preparation in the impregnation solution.

Duvetter et al. (2005) compared the passive osmotic infusion, vacuum-assisted infusion, and pressure-assisted infusion with the aim to improve the firmness of strawberry halves pretreated with PME and calcium chloride. The vacuum-assisted infusion was found to be the best method to accomplish an uptake of infusion solution and hence capable of improving the firmness of the strawberries (**Fig. 18 a-c**). The pretreated strawberry halves by vacuum-assisted infusion with PME and calcium chloride were subsequently subjected to thermal treatments (60 ℃ and 80 ℃) and pressure treatments (400 MPa and 550 MPa). For both the treatments, the pretreated samples were significantly firmer compared to the control sample (**Fig. 19a and 19b**). Fraeye et al. (2010) studied infusion of fungal pectin methylesterase and calcium prior to thermal, high pressure and thermal-high pressure processing of strawberries. Processing of strawberries caused a decrease in firmness, which was limited by infusion of PME and calcium chloride. PME was able to decrease the degree of methoxylation of pectin, accompanied by an increased cross-linking of the chains. During high pressure or combined thermal-high pressure processing, the degree of methoxylation of pectin in infused strawberries was even further decreased, probably due to a higher activity of the fungal PME under high pressure which was reflected in a very firm texture even after 40 minutes of processing. However, tissue damage was more severe in the combined thermal-high pressure process.

Guillemin et al. (2006) studied the penetration of PME by soaking under atmospheric pressure or vacuum-impregnation in apple cubes. The vacuum impregnation resulted in higher penetration of PME as compared to soaking under atmospheric pressure for 15 min or 1 hour treatment (**Fig. 20a**) and in a more homogeneous distribution of the enzyme in apple cubes (**Fig. 20b**). Vacuum-impregnation thus constitutes a valuable process for placing an enzyme in contract with substrate. It was concluded that the vacuum impregnation could be used to infuse exogenous enzyme to fruit pieces more rapidly and more homogeneously than soaking in order to improve the firmness of thermally treated fruits. Further, Guillemin et al. (2008) indicated that infiltration of the enzyme in apple cubes resulted in demethyl-esterification of pectins, combined with the increased concentration of calcium contribute to create new pectin ionic bonds leading to the strengthening of the tissue. Similarly, the higher mechanical rigidity of canned peaches and pasteurized strawberries due to infiltrated with PME and calcium was reported by Javeri et al. (1991) and Degraeve et al. (2003), respectively.

Buggenhout et al. (2006) studied the vacuum infusion, freezing, frozen storage and thawing conditions to minimize the texture loss of frozen strawberries. A significant texture improvement was observed when infusion of PME and calcium was combined with rapid or cryogenic freezing. During frozen storage, textural quality of product infused with PME/calcium and high-pressure frozen sample was maintained at temperatures below −8°C, whereas the texture of product frozen under cryogenic freezing conditions was only preserved at temperatures below −18°C. Thawing at room temperature was shown to be an appropriate method. Further, Buggenhout et al. (2008) indicated that the presence of PME and calcium in an osmotic solution during osmotic dehydration of strawberries resulted in decrease in weight reduction, which was correlated to a small positive effect on the net uptake of sugars and depression of the initial freezing point during osmodehydrofreezing. The added PME and

calcium reduced the drip loss upon thawing and was found to positively influence the volume and shape of the thawed samples.

6. Infusion of probiotics

The development of novel probiotic foods have been focused mainly on dairy products. The consumption of these products showed a progressive increase in the last decade due to changes in habits and trends of consumers attracted by their benefits. Nowadays, the development of fruits and vegetables with probiotics content is of high interest for probiotic-food consumers (Puente et al., 2009). Fuller (1989) gave a widely accepted definition of probiotics as "live microbial feed supplements which beneficially affect the host by improving its intestinal balance" the beneficial effects and applications of probiotics are the focus of active research and have been reviewed by many researchers (Reuter et al., 2002; Koop-Hoolihan et al., 2001; Sanders et al., 2001; Goldin et al., 1998; Reddy et al., 1998). Probiotic microorganisms belong to different microbial genera, *Lactococcus, Lactobacillus, Bifidobacterium, Saccaromyces, Bacillus, Entercoccus, Pediococcus* or *Leuconostoc* (Reuter et al., 2002; Prado et al., 2008; Shah., 2007; Fooks., 2002; Gomes., 1999). The most frequent bacteria commercially used dairy products belong to the *Lactobacillus* and *Bifidobacterium* species, although *Streptococcus thermophilus* and *Saccharomyces boulardii* are also available in certain milk products (Rastall et al., 2000).

Rodrıguez (1998) evaluated the effect of vacuum impregnation on the penetration of different microorganisms (*Saccharomyces cerevisiae, actobacillus acidophilus and Phoma glomerata*) into apple. A comparison of microbial counts of fresh apple, apple treated under atmospheric or vacuum conditions indicated that the lower pressure resulted in higher impregnation of microorganisms. The simple soaking also was found to render a significant increase in microbial counts, which highlighted the fact that capillary force and superficial

adherence are the important factors that cannot be neglected in any modelling approach of immersion and impregnation operations. The model proposed by Roa et al. (2001) was used for predicting microorganism incorporation into vegetable tissues. The authors simplified the model developed by Fito (1994) by accomplishing direct experimental determination of the volumetric fraction of sample occupied by the impregnating solution as a result of hydrodynamic mechanism.

Maguina et al. (2002) investigated the fortification of apple cylinders with *Bifidobacterium* spp. by applying vacuum impregnation and the microorganism was incorporated at levels higher than 10^7cells/g. Maintaining the viability, stability and functionality of probiotics not only during processing but during storage is also essential to deliver the health benefits of these microorganisms to consumers (Mattila-Sandholm et al., 2002; Saarela et al., 2002). Viability evaluation of *Bifidobacterium* spp. in apple pieces stored in anaerobiosis at 4°C for 12 days revealed that viable populations decreased only by one-log cycle after the sixth day, and remained in that level until the end of storage. The vacuum impregnated apple samples stored for six days showed the highest sensory scores for colour, odour and flavour, while control fruit pieces had less acceptability.

Betrot et al. (2003) studied the beneficial effects of probiotics enriched dried fruits by vacuum impregnation. Apple cylinders were impregnated with commercial apple juice containing *Saccharomyces cerevisiae* and whole milk containing 10^7 or 10^8 cfu/ml of *Lactobacillus casei* (spp. Rhamnosus). Impregnated apple samples contained around 10^7 CFU/g of each microorganism. Dendritic structures observed in the intercellular space confirm that gas was replaced by impregnating liquid. The cells of *S. Cerevisiae* and *L. casei* were imbibed in dentritic structures in the intercellular space of apple tissues (**Fig. 21a and 21b).** Enriched air dried apples stored at room temperature for two months showed that the

concentration *L. casei* in dried product was greater than 10^6 CFU/g, which was very similar to the levels usually found in commercial probiotic dairy products.

Puente et al (2009) evaluated the viability of a probiotics strain (*Lactobacillus rhamnosus)* inside a food matrix (Granny smith apple) using vacuum impregnation.. Micrographs revealed that the probiotics impregnate penetrated inside the fruit structure and were uniformly distributed in the sample. Even after storage for a period of 60 days the probiotics counts practically did not change (10^9 CFU/g) in frozen samples. The colour of frozen apples was reported to be more stable than refrigerated product.

Similarly, Ortiz et al. (2002) used the vacuum impregnation to fortify guava with *Bifidobacterium spp.* (400 mmHg, 5 min). Impregnated guava pieces contained around 10^7 CFU/g. Counts of Bifidobacterium spp. decreased in 3 log cycles after 12 days of storage at 5 °C because no special anaerobic packaging was used.

7. Infusion of flavours

The food industry often utilizes flavor-enriched, semifinished food products as ingredients in more complex preparations. There are no methods to produce minimally processed food items to which flavorings have been added. Comandini et al. (2010) studied the incorporation of green apple aroma in apple sticks using various techniques such as atmospheric pressure impregnation, vacuum impregnation, ultrasound and the combination of vacuum impregnation and ultrasound. An isotonic solution of fructose containing ascorbic acid and green apple flavoring was used for impregnation of apple sticks. Vacuum impregnation and combined vacuum and ultrasound resulted in the highest aroma enrichment at 5.0 min of treatment (**Table 2**) due to the higher fraction of isotonic solution that penetrated inside the apple sticks by a hydrodynamic mechanism. Ultrasound treatment was found to be similar to the infusion at atmospheric pressure.

8. Conclusion

A significant advancement has been made in impregnation of bioactive constituents in solid food matrix using various techniques. This indicates that there is an ample scope for further research and development in this area for the development of nutritionally enriched solid foods. The enrichment of fruits and vegetables with minerals, vitamins or other physiologically active compounds can be good choice to develop functional foods. Osmotic treatment was found to be a feasible method to incorporate bioactive compounds in food without altering its structure. Application of ultrasound or pretreatment with vacuum resulted in enhanced infusion of bioactive components in foods. The impregnation behaviour and fruit and vegetable matrices have profound effect on health benefits. The process parameters may be selected depending on the extent of bioactive compound or osmotic solute infusion required for product development. Fruit and vegetable matrices will certainly be an important research and development area for future functional food markets. These infusion techniques may be useful for the infusion of other nutraceuticals like pigments, dietary fibre, cryoprotectants and antimicrobials for the development of novel food products. Nutraceuticals obtained as by-products of food industry such as reservatol from peanut or grape skin can be beneficial bioactive constituents for impregnation. Knowledge to assess the effects of these matrices in the body is also a highly desirable goal.

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Table 1: Work carried out by various researchers on osmotic dehydration assisted impregnation.

Food Matrix	Osmotic Agent	Impregnated compound	Reference
Probiotics			
Apple, guava	Isotonic sucrose solution, commercial apple juice, whole milk, grape must at pH-5.0	*Saccharomyces cerviviae, Lactobacillus acidophilus, Phoma golmerata, Bifidobacterium spp., Saccharomyces cervisiae, Lactobacillus casei, Lactobacillus rhamnosus*	Maguina et al., 2002; Ortiz et al., 2002 ; Betroet et al., 2003 ; Puente et al 2009
Minerals			
Apple, egg plant, oyster, mushroom, carrot, melon, papaya	Isotonic glucose solution Isotonic Sucrose solution Calcium chloride	Calcium lactate, calcium gluconate, calcium and znic	Alzamora et al.,2001; Anino et al., 2002; Gras et al., 2002,Tapia et al., 2003; Gonzalez et al.,2002,Ortiz et al., 2003; Perez-Lopez et al., 2002; Barrera et al., 2009
Grape Phenolics			
Model food, potato, apple and banana	Grape seed extract (Sucrose, NaCl and glycerol)	Phenolics	Rozek et al., 2007; 2008; 2009; 2010a; 2010b.
Vitamins			
Potato tuber, pears, apple	20% Honey 55° Brix, 20°Brix 30, 40 and 50° Brix	10% Ascorbic Acid A-tocopherol acetate, free α-tocopherol, water soluble α-tocopherol acetate Iron,B-Carotene	Hironaka et al. 2011 ; Lin et al. 2006; Barrera et al. 2004; Santacruz-Vazquez et al. 2008
Enzyme			
Apple cubes, strawberries	- 60% glucose, fructose, sucrose and raftilose	Pectinmethylesterase	Gullemin et al. 2008; 2006; Buggenhout et al. 2008, 2006; Fraeye et al. 2010
Antioxidant			
Apple	0.3% high in flavonoids apple peel extract	Quercetin glycosides	Schulze et al. 2012
Fresh coconut	Sucrose, NaCl and their combination	Curcuminoids	Bellary et al. 2011; 2012
Flavour			
Apple	Isotonic solution of fructose	Green apple aroma	Comandini et al 2010

Table 2: Relative amounts of the overall flavouring obtained with different impregnation techniques and treatment times

Treatment	Time		
	2.5min[b]	**5.0min[a]**	**12.5min [a]**
AI[b]	0.05±0.04	0.23±0.04	0.24±0.03
USI[b]	0.19±0.04	0.24±0.04	0.23±0.03
VUSI[ab]	0.20±0.04	0.36±0.03	0.27±0.03
VI[a]	0.21±0.06	0.35±0.06	0.36±0.03

AI- Atmospheric pressure, VI- Vacuum application, USI-Ultrasound treatment, VUSI-Vacuum Ultrasound treatment Values are means ± standard deviations (n=3). a, ab ,b Different letters in rows show statistically significant differences between treatments ($p < 0.05$). a, b Different letters in columns show statistically significant differences between times ($p < 0.05$) (Comandini et al .,2010).

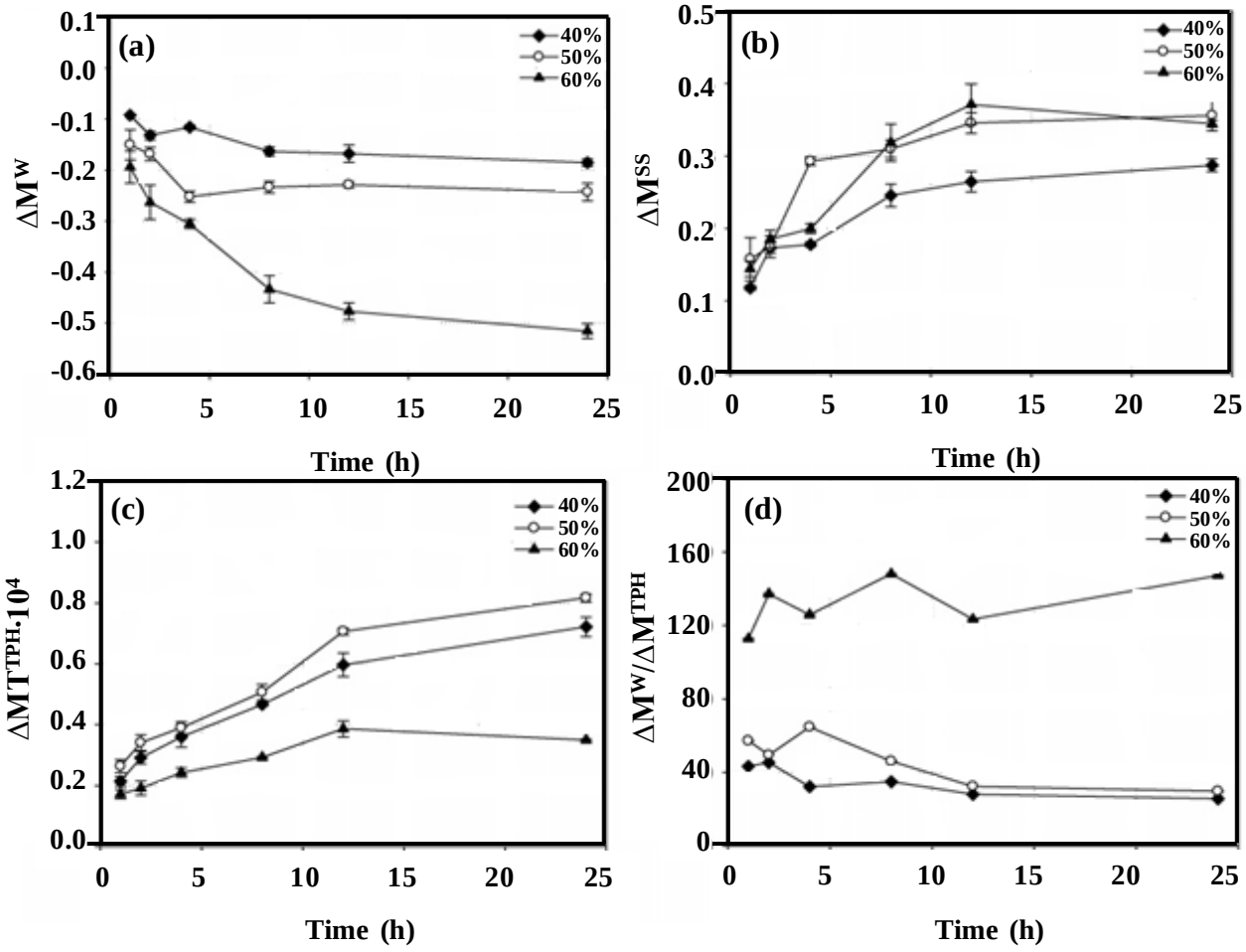

Fig. 1: (a) Mass changes of water (ΔMw), (b) gain in soluble solids (ΔMSS), (c) total phenolics (ΔMTPH), and (d) ratio of water mass changes to gain in total phenolics (ΔMw/ΔMTPH) during OD with red grape must. Mass fraction of soluble solids in the red must was adjusted to 40, 50 and 60%. (Rozek et al., 2007a)

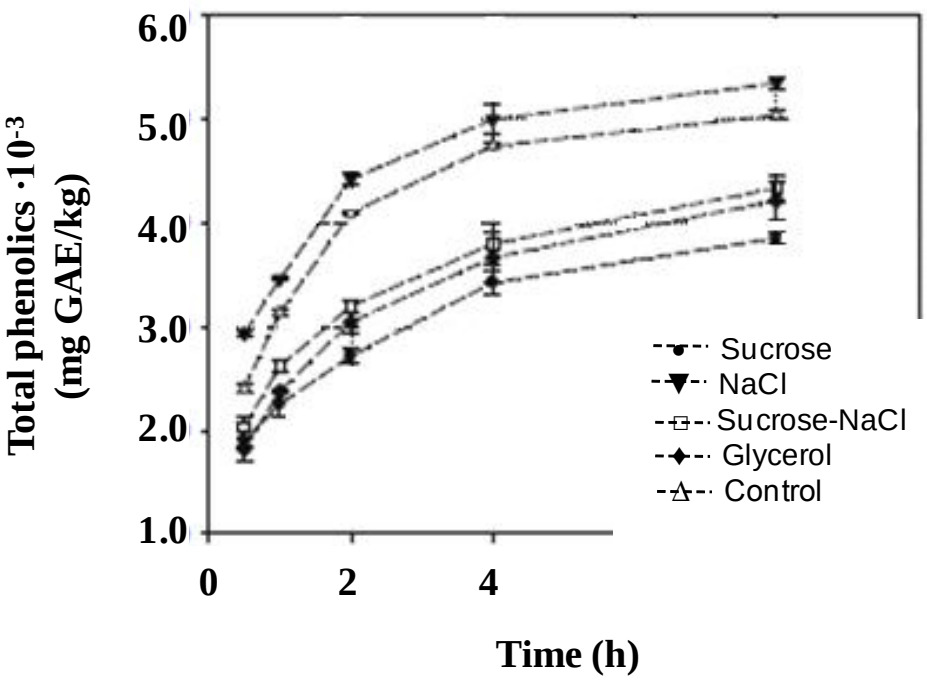

Fig. 2: Total phenolic content in osmo-treated food determined by Folin-Ciocalteau during osmotic treatment with several osmo-active solutes (Rozek et al., 2009).

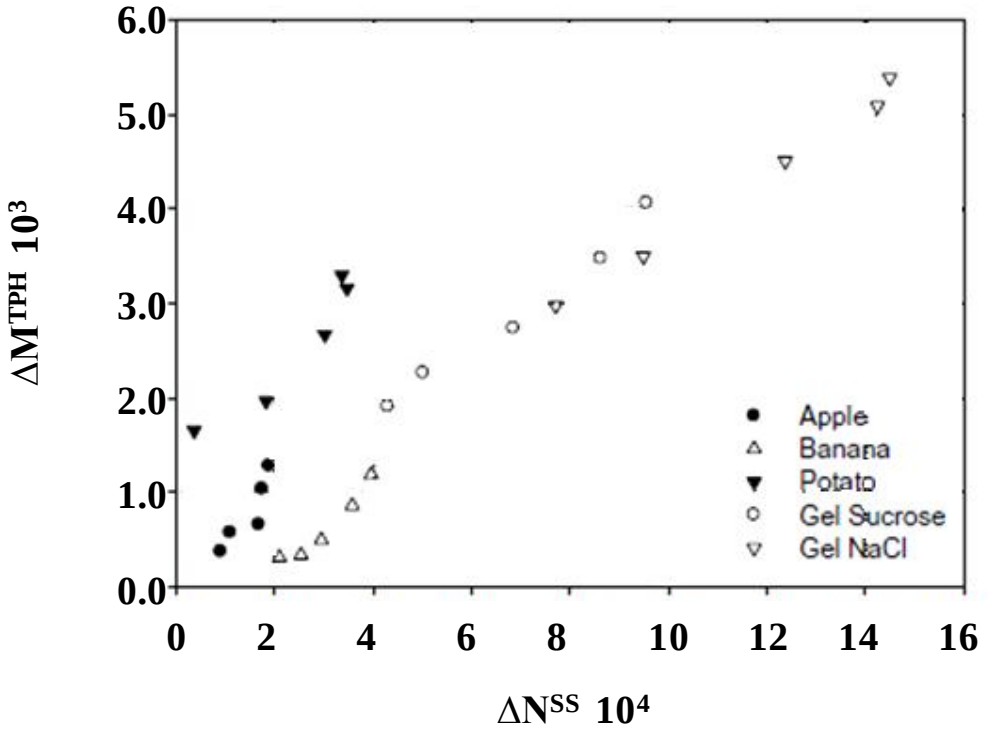

Fig. 3: Mass change during osmotic treatment of total phenolics (ΔTPH) versus solid gain in moles (Rozek et al., 2010a).

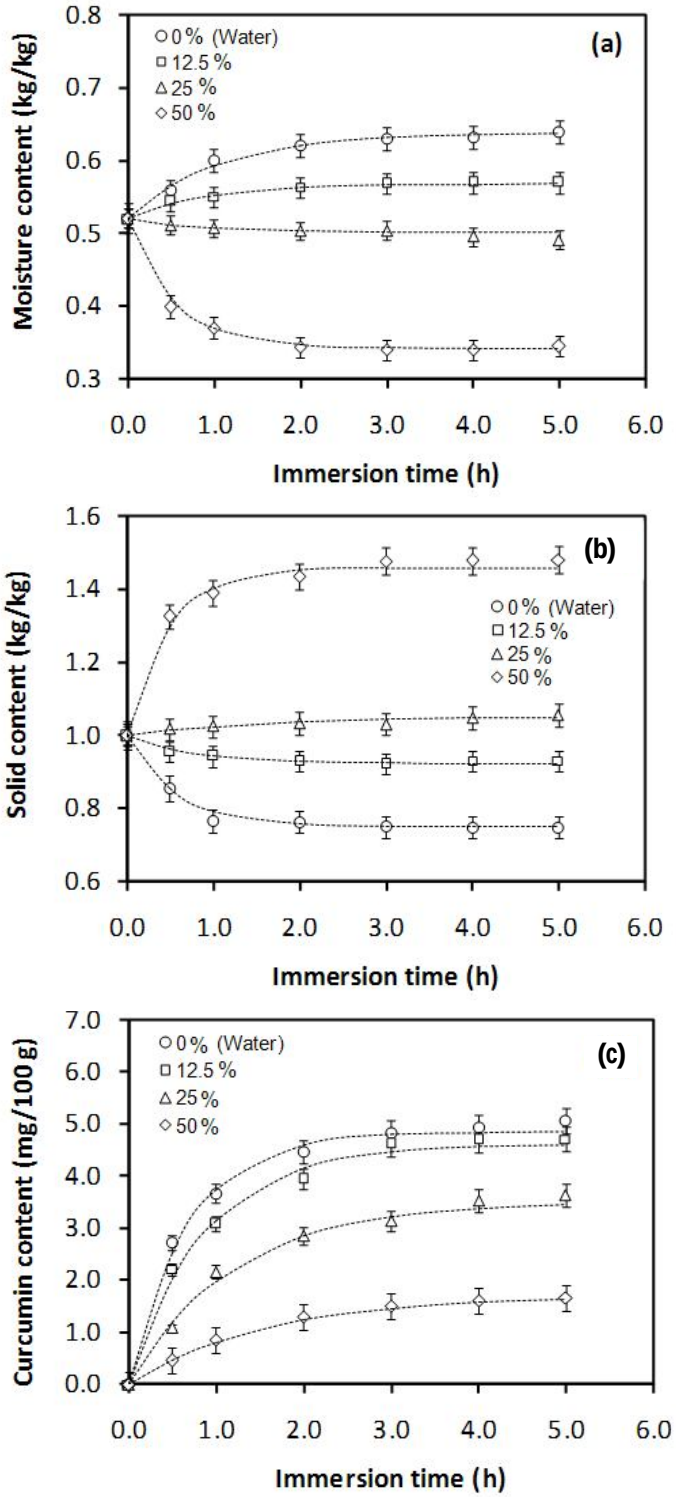

Fig. 4: Variation of (a) moisture, (b) solid and (c) curcuminoids content during osmotic dehydration assisted impregnation of coconut sample for various concentrations of osmotic solution. (Bellary et al., 2011)

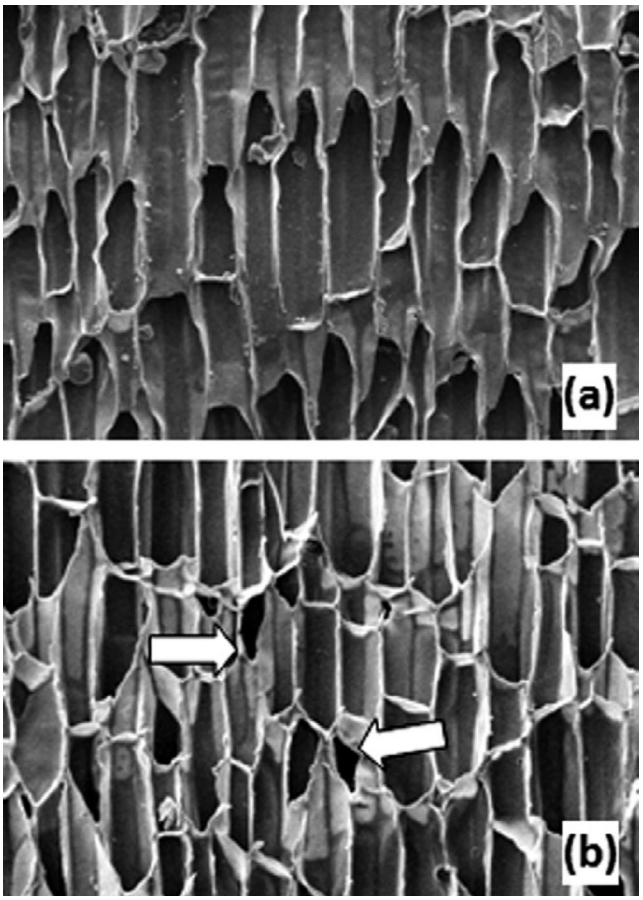

Fig. 5: Microstructures of (a) untreated coconut and (b) coconut subjected to ultrasound. Arrows indicate the cell breakage.. (Bellary et al., 2011)

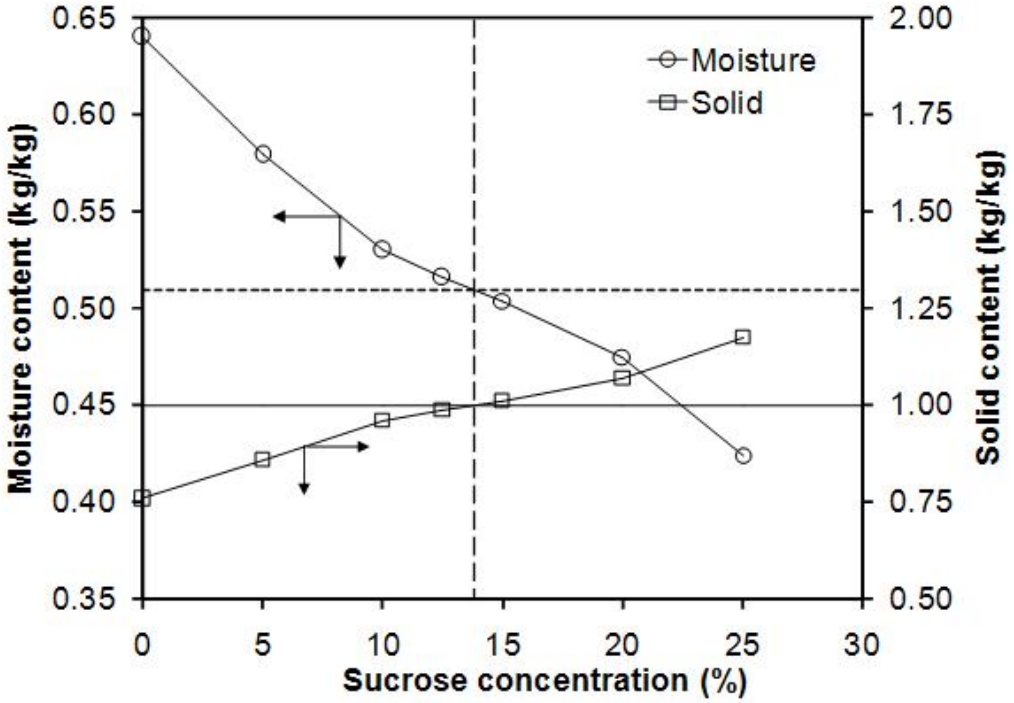

Fig. 6: Variation in moisture and solute content of coconut sample with surrounding solution concentration. The total soluble solid in coconut was 13.5%. (Bellary and Rastogi, 2012)

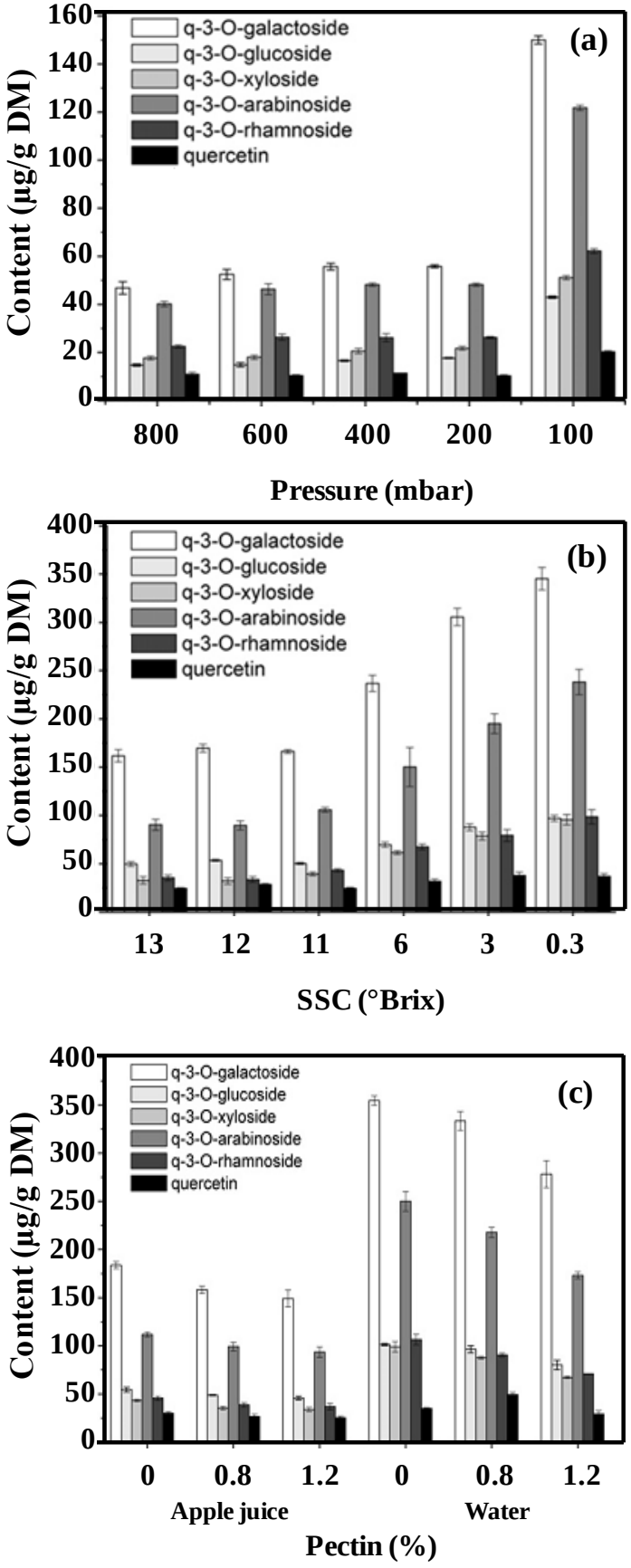

Fig. 7: Quercetin aglycone and quercetin glycoside content ± SD after VI of apple slices (cv. Jonagold) depending on (a) vacuum pressure (b) soluble solid content (c) apple pectin VI solutions (n = 3) (Schulze at al., 2012).

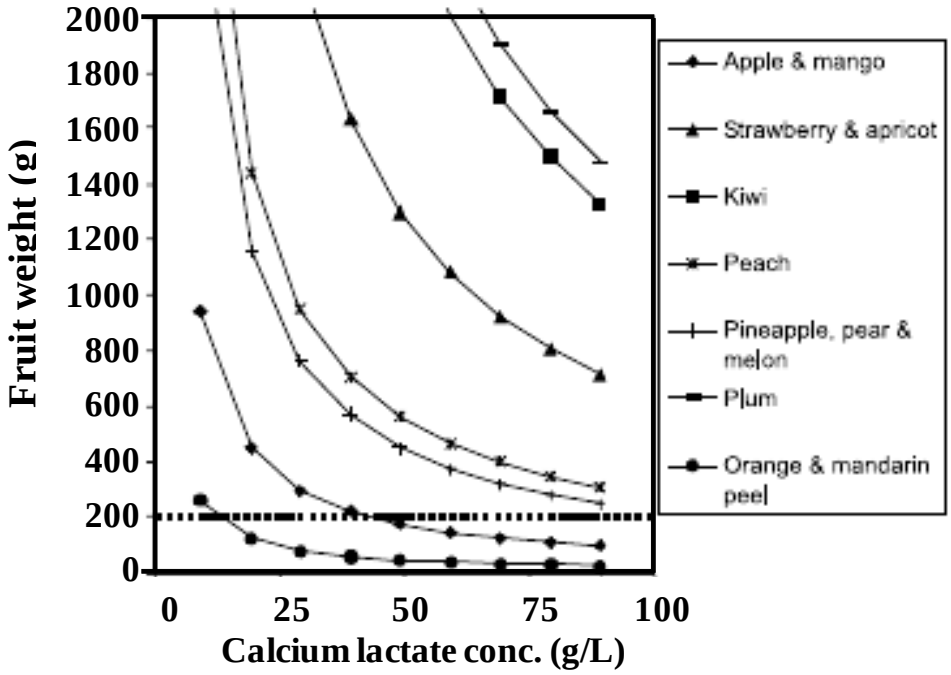

Fig. 8: Weight of some functional food (fruit products) containing 25% of the recommended daily intake(RDI) as fuction of calcium conceration in impregnation solution (Fito et al., 2001).

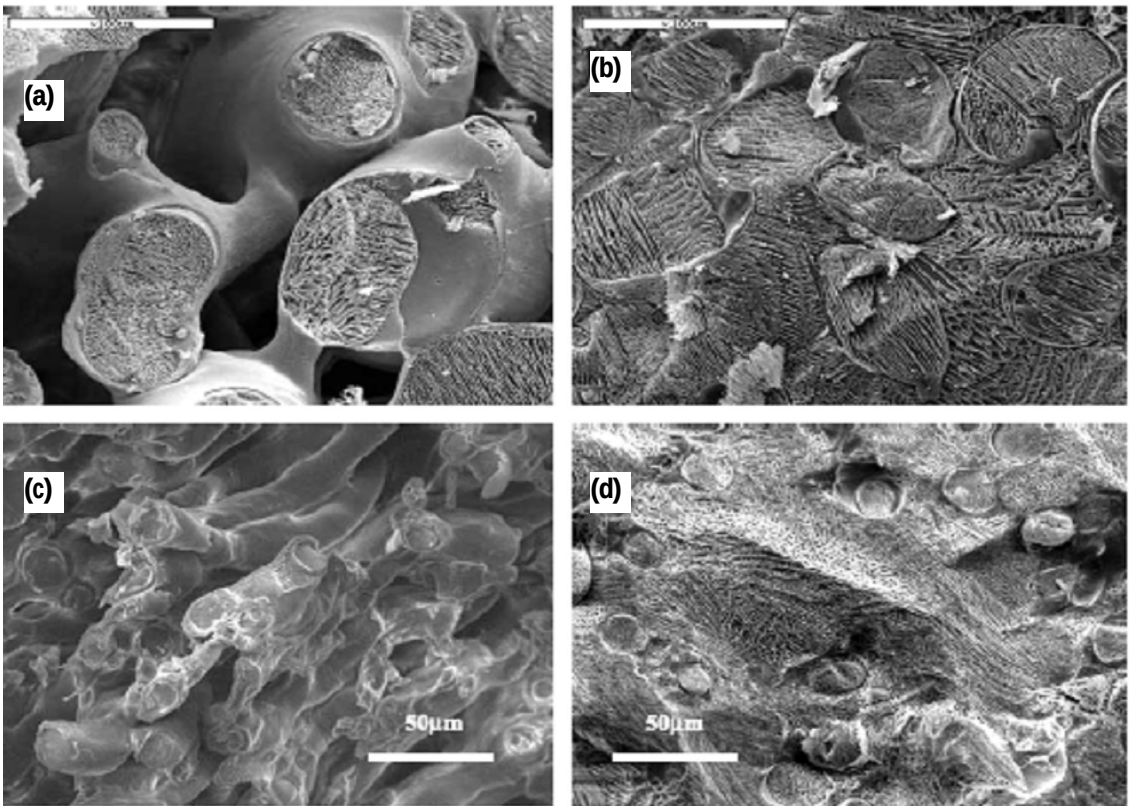

Fig. 9: Cryo-Sem micrographs of orange peel(a) and (b) and egg plant (c) and (d) before (a) and (c) and after (b) and (d) vacuum impregnation with an isotonic solution. The big intacellular spaces (IS) in the tissues appear completely flooded by the external solution in both cases after VI operation. Thus vaccum impregnation method is a good choice to produce fresh cut fruits or vegetables enriched or fortified with physiologically active component. (Fito et al., 2001)

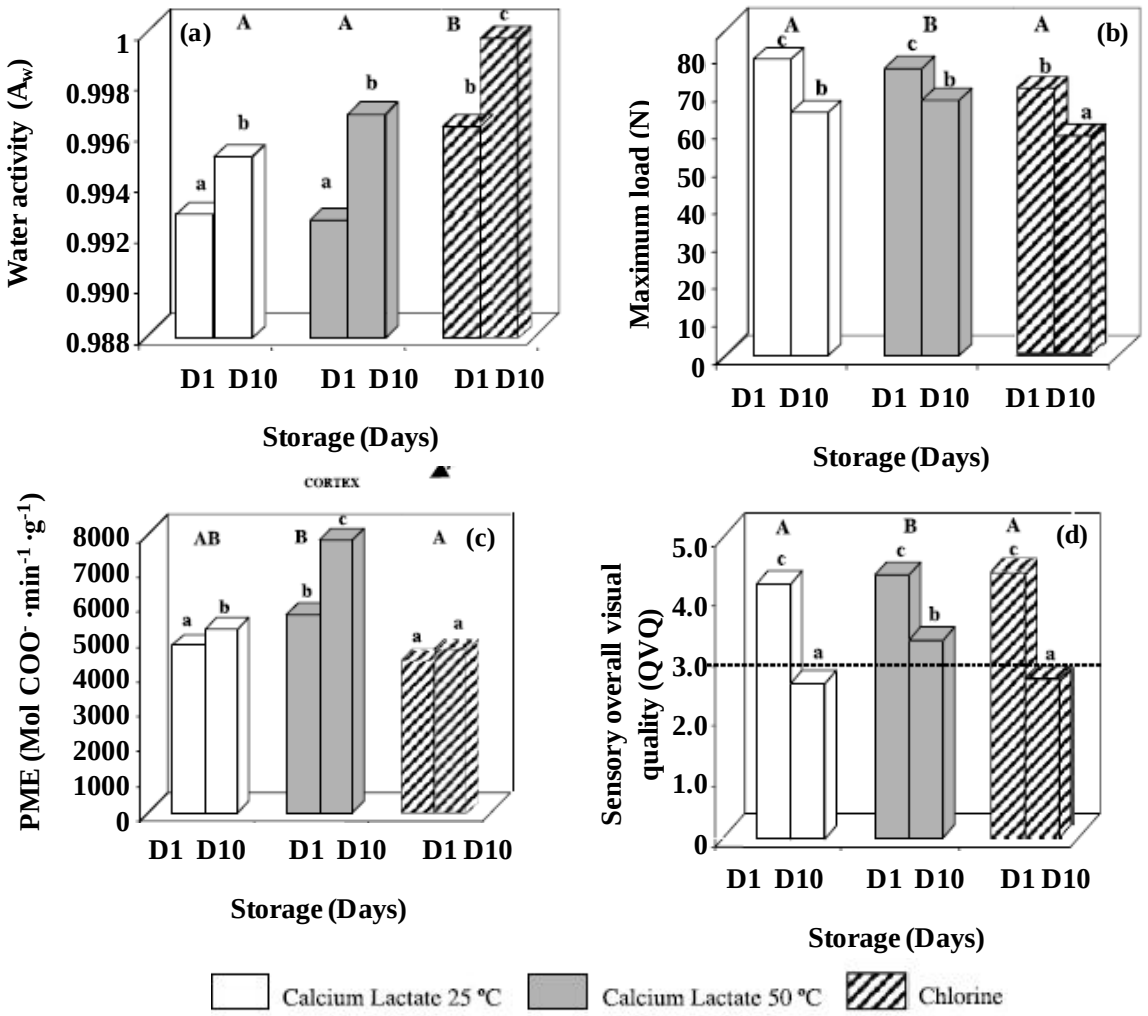

Fig. 10: (a) Water activity, (b) maximum load, (c) water activity and (d) sensory analysis after treatment (day 1) and at the end of the storage (day 10) in sliced carrots treated with chlorine (120 mg L^{-1}), calcium lactate (15 g L^{-1}) at 25°C and calcium lactate at 50°C (Rico et al., 2007).

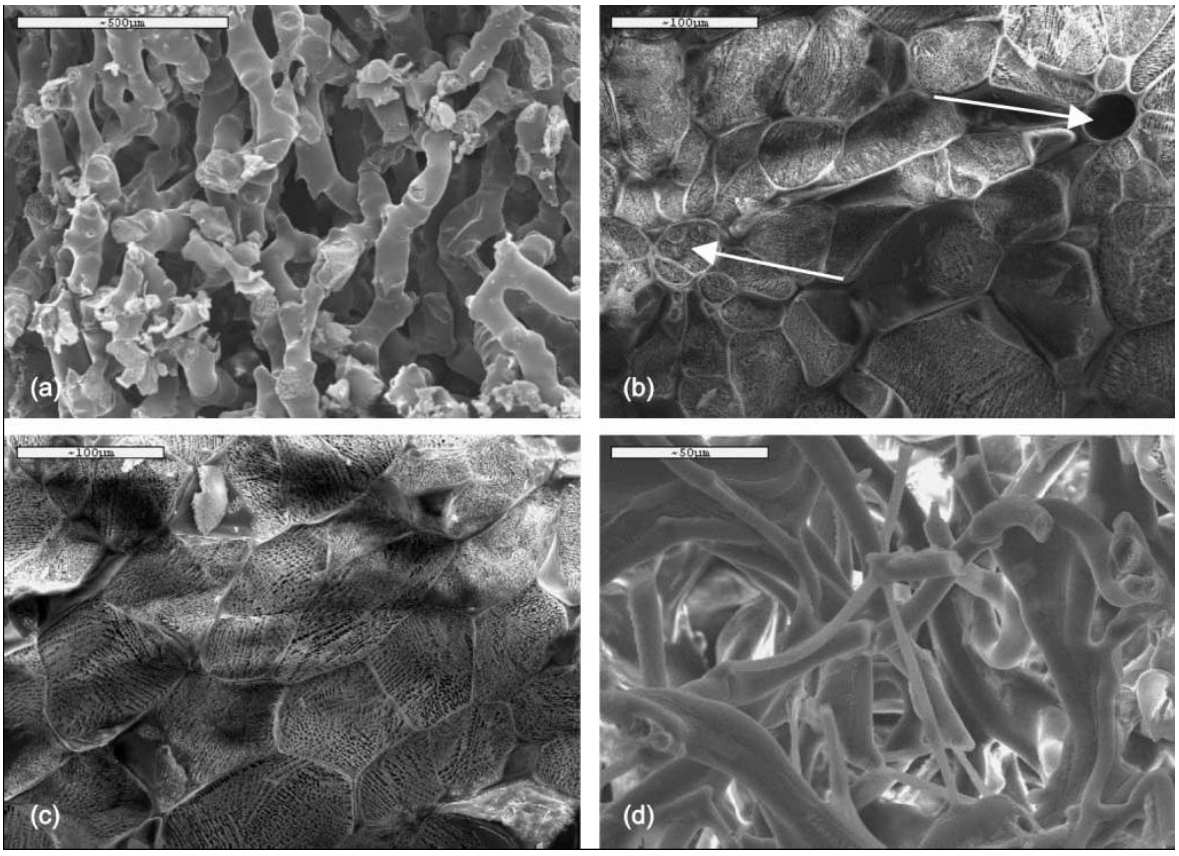

Fig. 11: (a) Cryo-SEM micrographs of eggplant, (b) carrot xylem (arrows: tracheary elements), (c) carrot parenchyma and (d) and oyster mushroom (Gras et al., 2003).

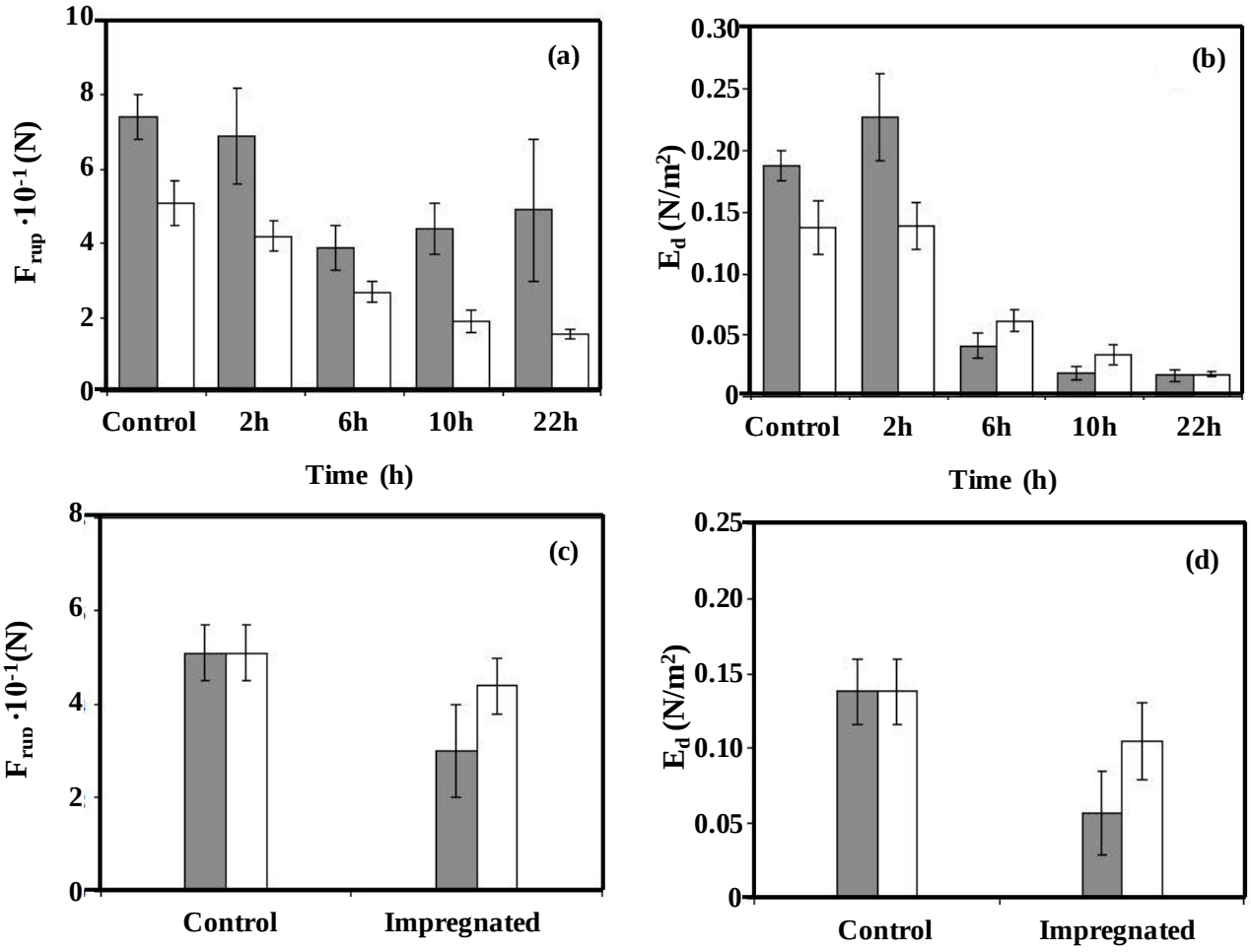

Fig. 12: Effect of calcium incorporation on the rupture force (F_{rup}) and mean values of the modulus of deformability (E_d) of apples subjected to impregnation in the isotonic glucose solution with and without Ca^{2+}: (■) with calcium; (□) without calcium; (a, b) atmospheric impregnation and (c, d) vacuum impregnation (Anino et al., 2006)

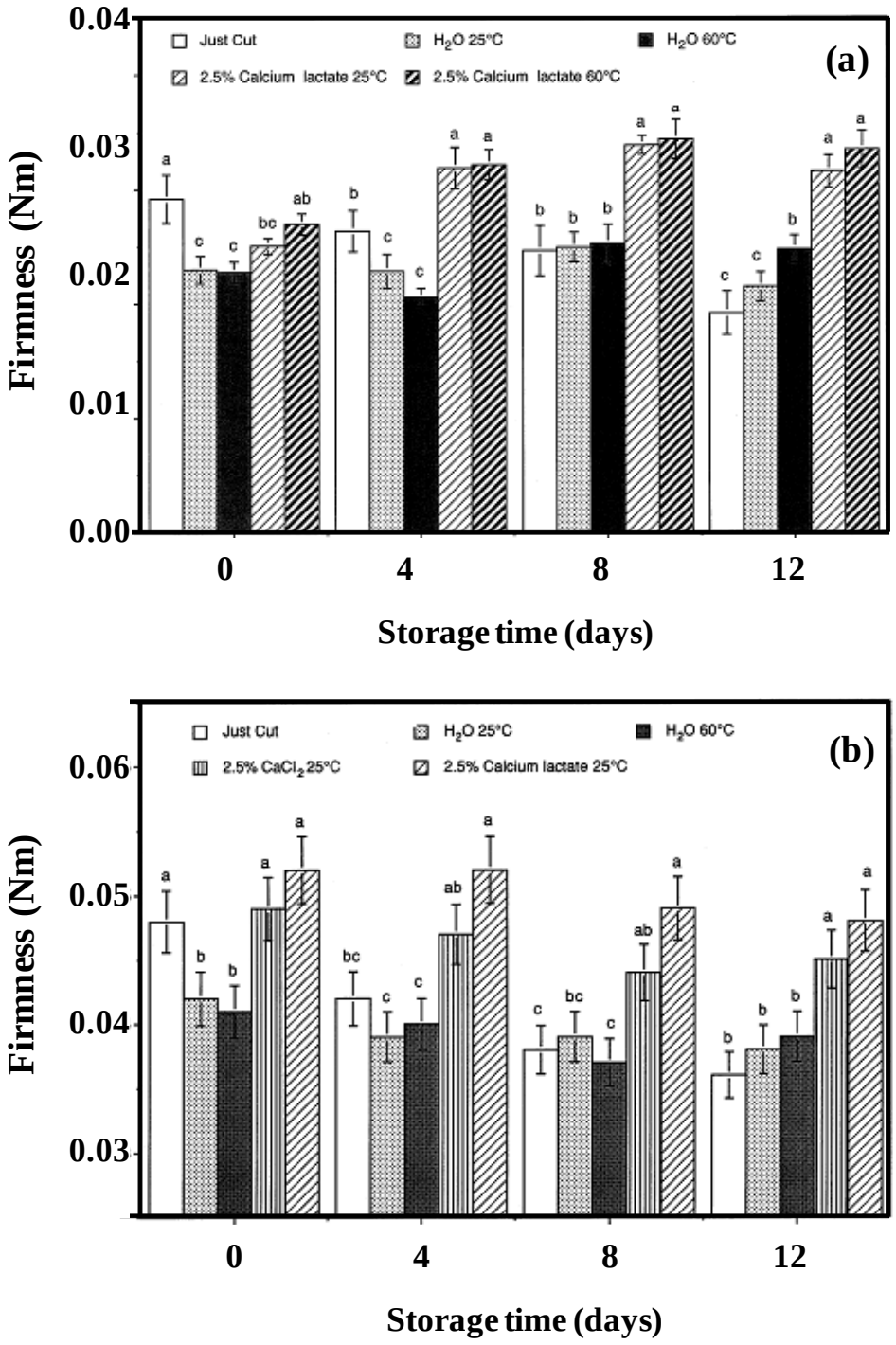

Fig. 13: Firmness (Nm) of fresh-cut cantaloupe dipped in water or (a) 2.5% calcium lactate, (b) 2.5% calcium chloride for 1 min at 25 or 60°C, and stored under air at 5°C and 95% RH (Luna-Guzman et al., 2000).

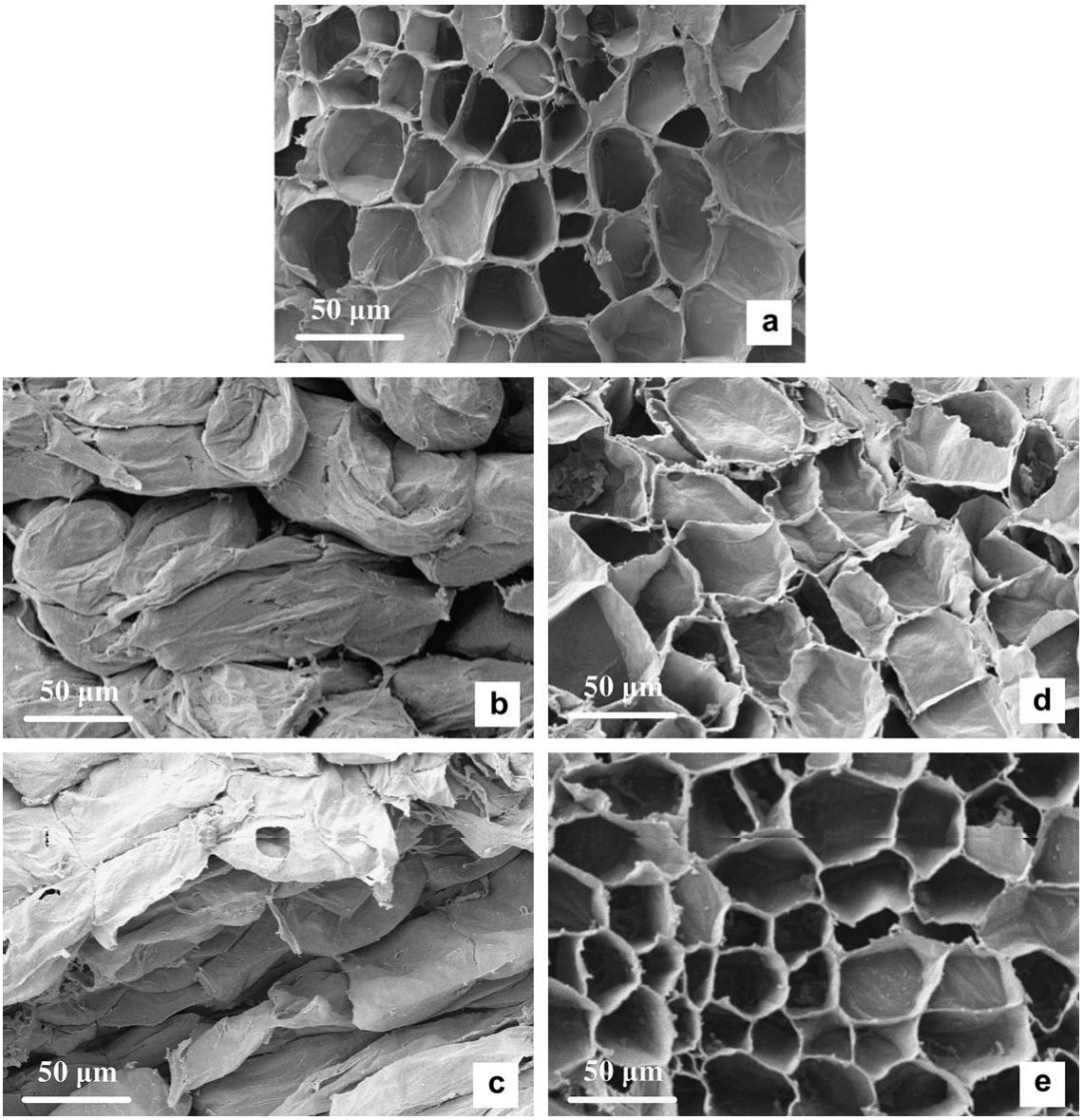

Fig. 14: Microstructures of carrot samples: (a) fresh; (b) thermal processed (105 _C, 10 min); (c) thermal processed (105 _C, 10 min) with combined pretreatment (d) pressure assisted thermal processed (700 MPa, 105 _C, 5 min); and (e) pressure-assisted thermal processed (700 MPa, 105 _C, 5 min) with combined pretreatment. (Rastogi et al., 2008)

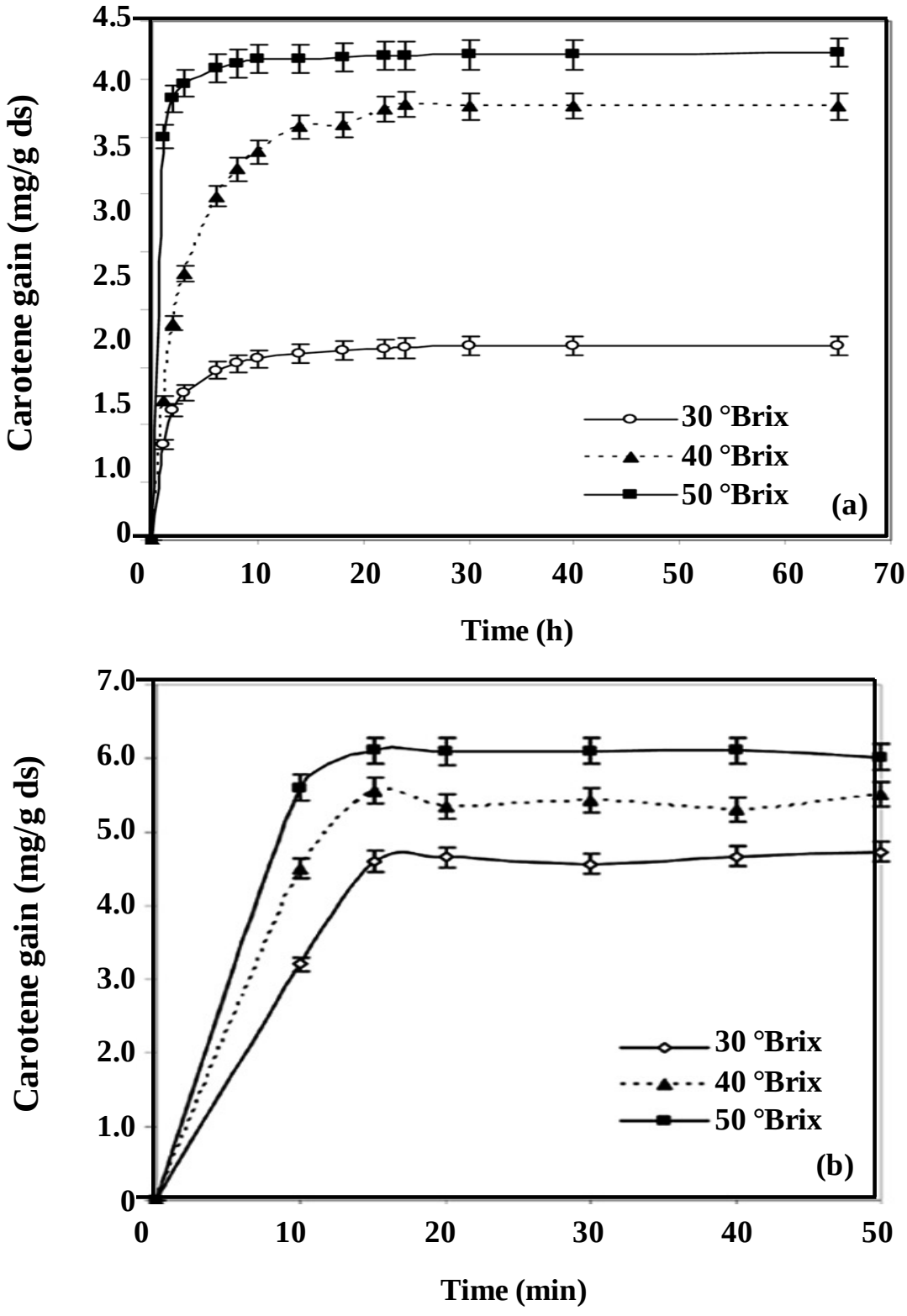

Fig. 15: Carotene gain during osmotic dehydration of apple slices with different osmotic concentrations without (a) vacuum and (b) with pulsed vacuum (Santacruz-Vazquez et al., 2008)

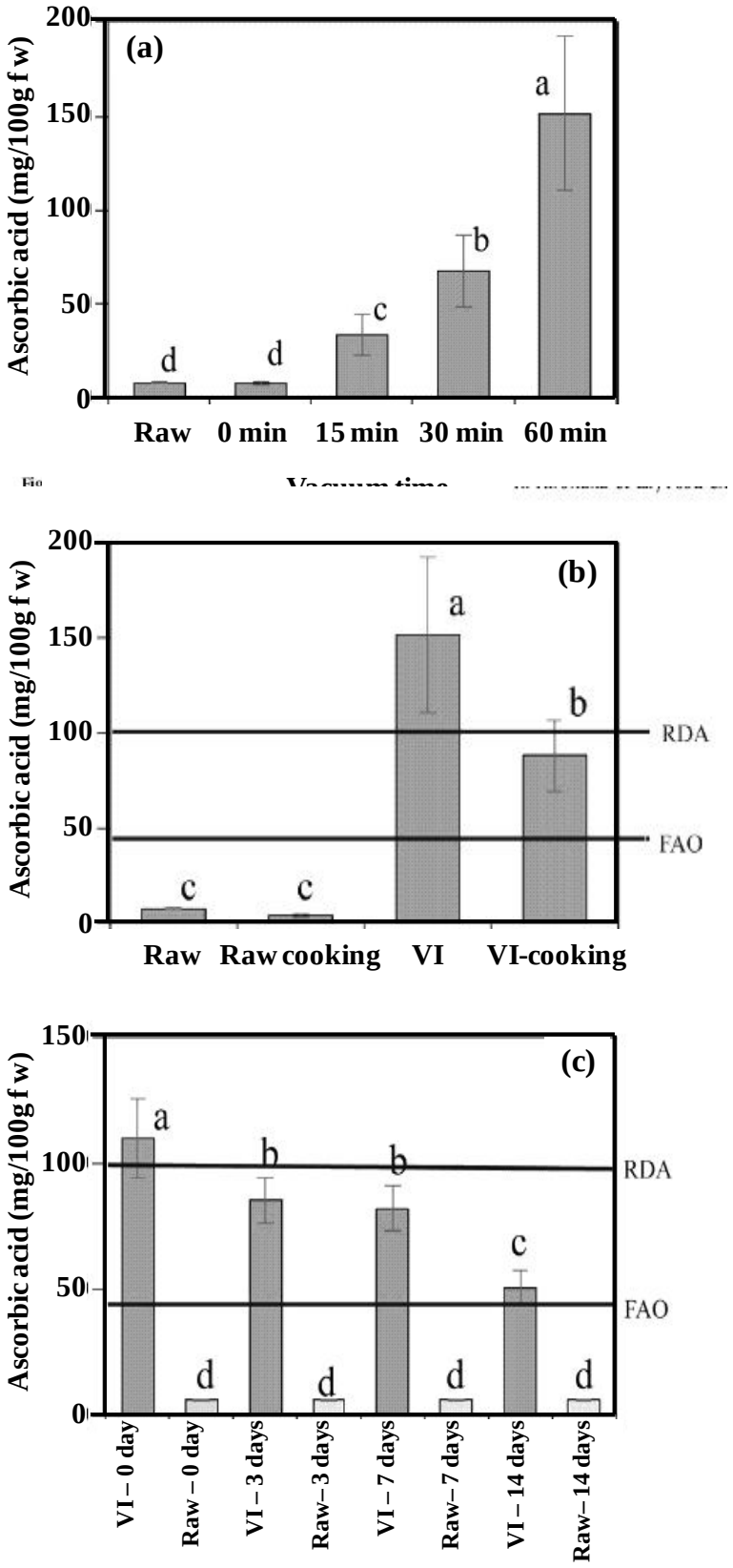

Fig. 16: (a) Effect of vacuum time, (b) steam-cooking and (c) storage on ascorbic acid content of whole potato tuber. (Hironaka et al., 2011)

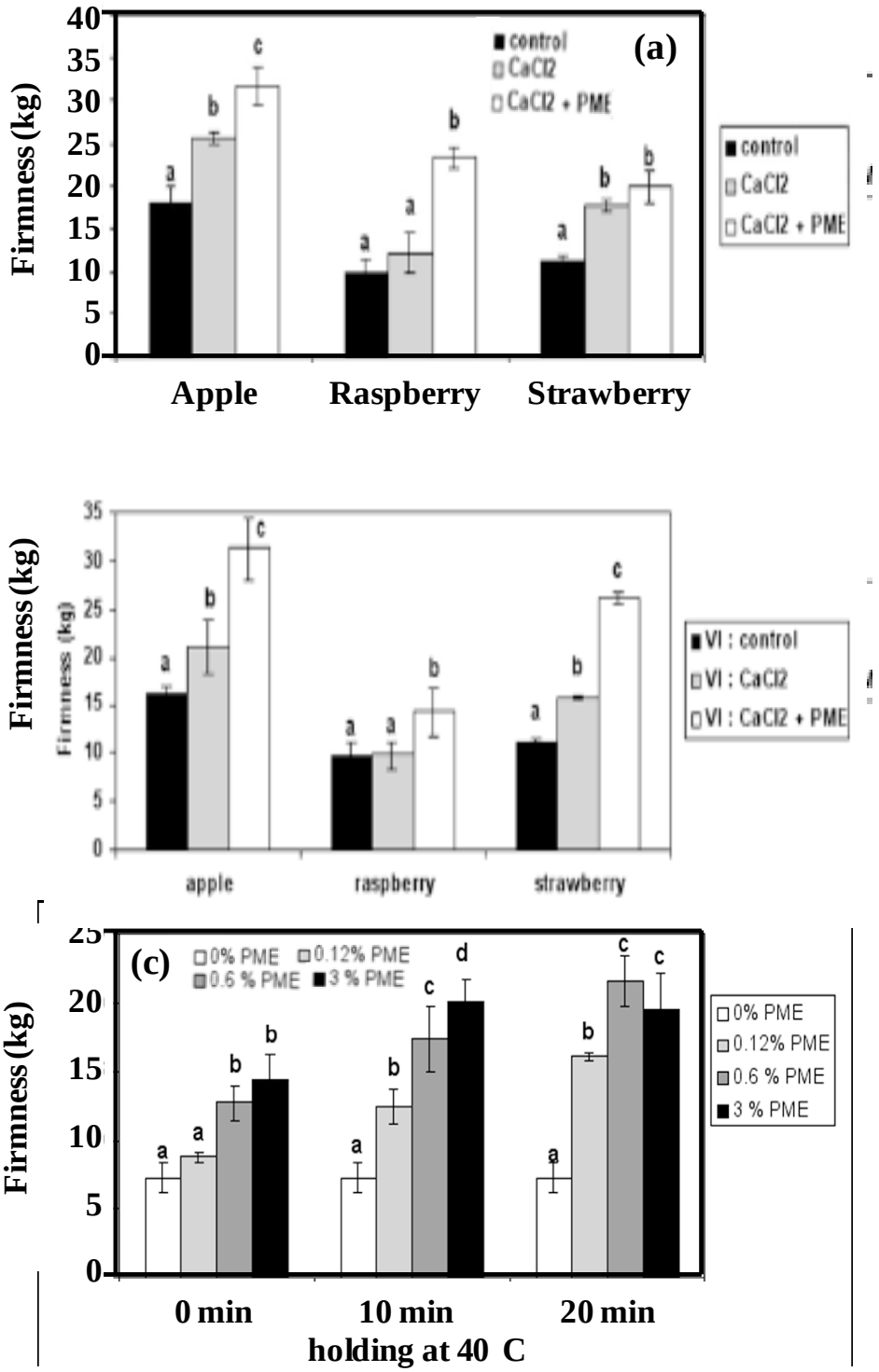

Fig 17: Firmness of pasteurized strawberries halves, apple cubes and whole raspberries treated by a classical (A) or a vacuum-assisted (B) infusion procedure and (C) Firmness of pasteurized strawberries halves pretreated by vacuum impregnation. Means with different letters indicate significant differences ($P < 0.05$) between treatments (Degraeve et al., 2003)

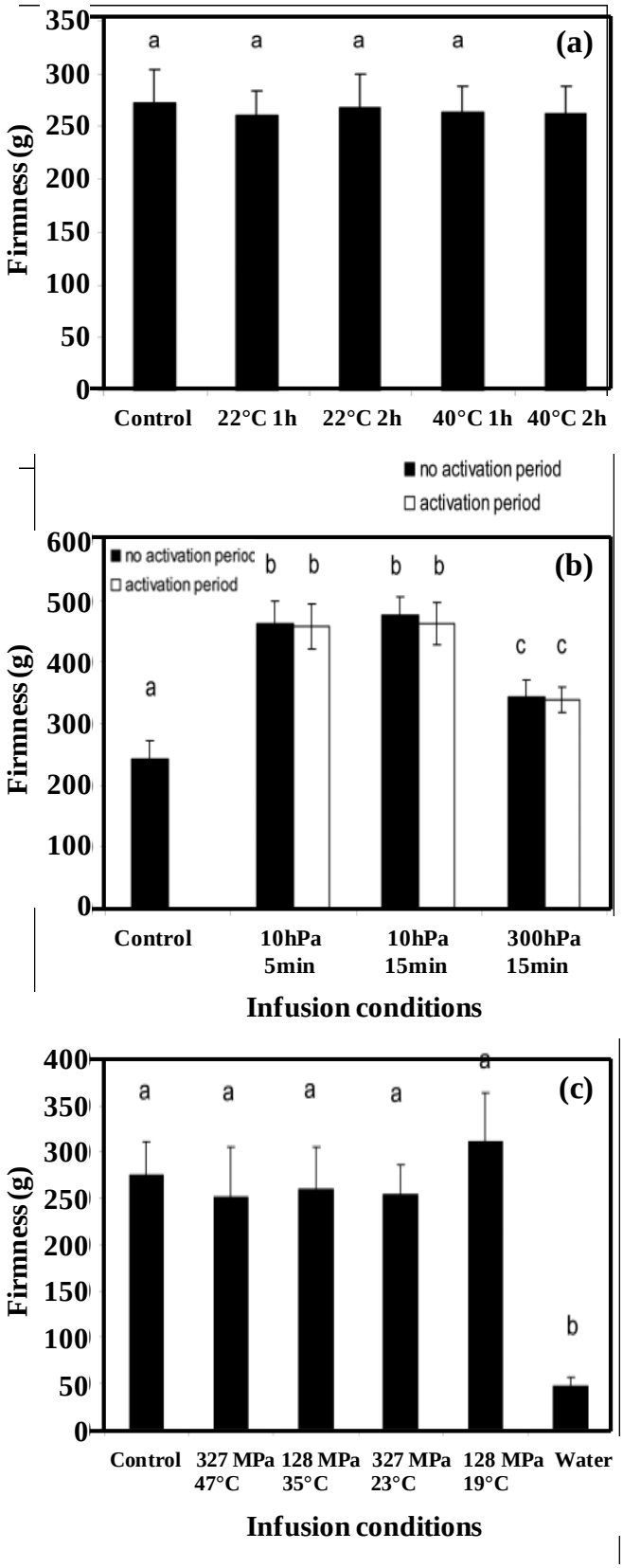

Fig. 18: Firmness of strawberries following (a) passive osmotic infusion, (b) vacuum assisted infusion and (c) pressure-assisted infusion. Control = non-infused strawberries. Means with the same letter (a) indicate there is no significant difference (Tukey's HSD test: $P < 0.05$) between treatments (Duvetter et al., 2005).

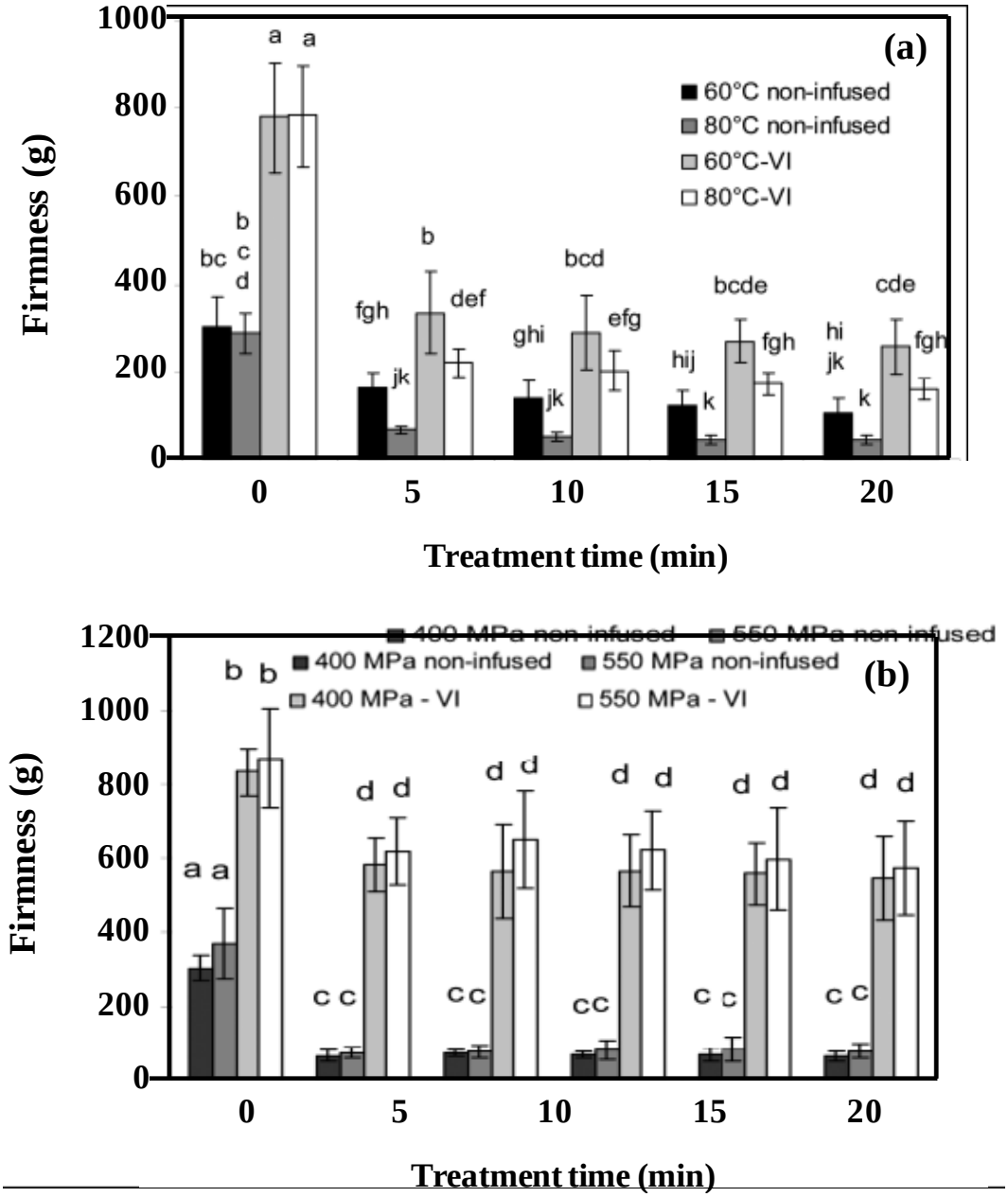

Fig 19: Firmness of non-infused and vacuum-infused strawberries after (a) thermal treatment at 60 °C and 80 °C as well as (b) pressure treatment at 400 MPa and 550 MPa. Means with the same letter (a-k) indicate there is no significant difference (Tukey's HSD test: $P < 0.05$) between vacuum-infused or non-infused, treatment temperature, and treatment time (Duvetter et al., 2005)

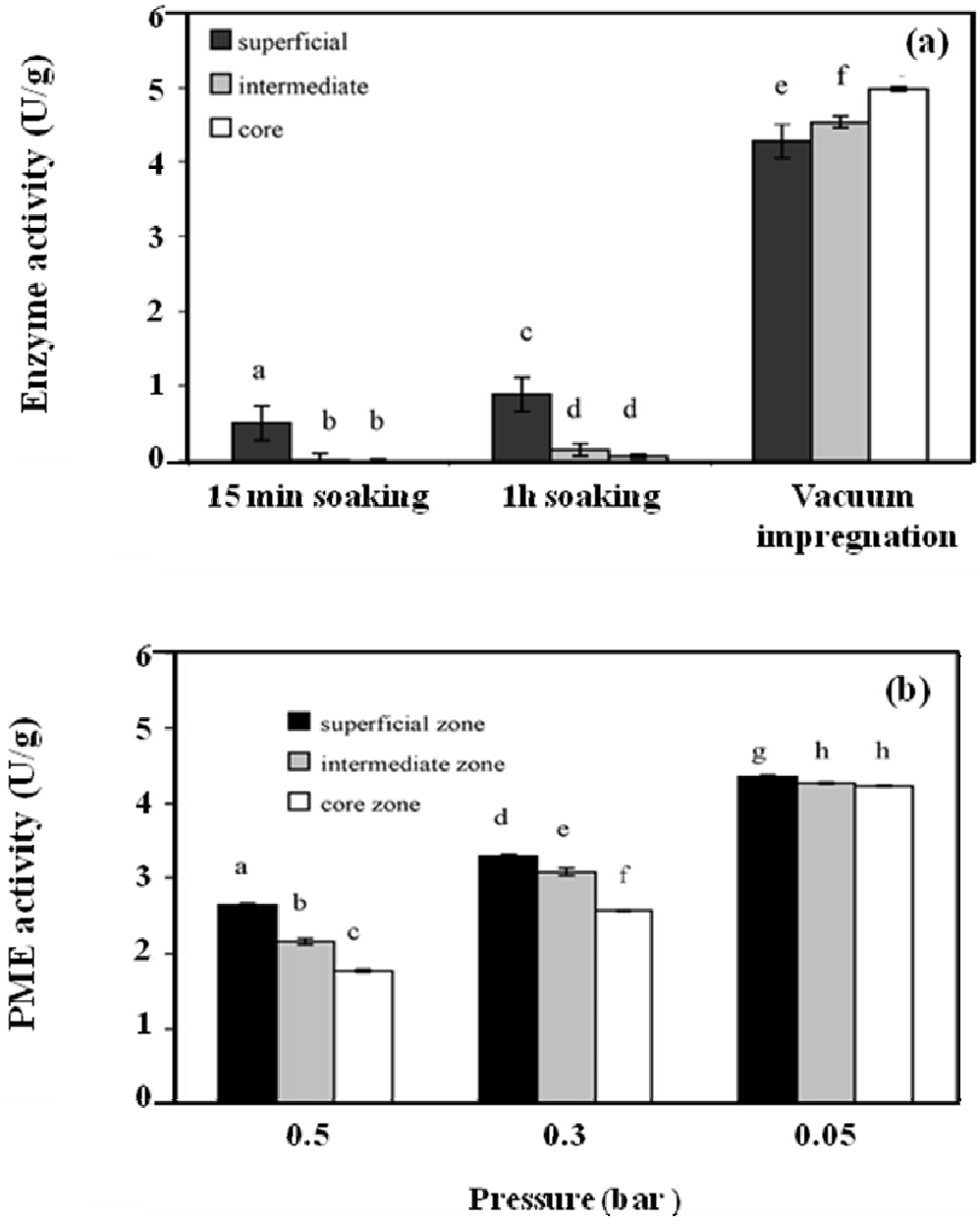

Fig 20: (a) Distribution of PME activity in infused and vacuum-impregnated apple cubes; (b) effect of vacuum pressure on distribution of PME activity in vacuum impregnated apple cubes. Means with the same letter are not significantly different at $P < 0.05$ as determined by Fisher's least significant difference procedure (Guillemin et al., 2006).

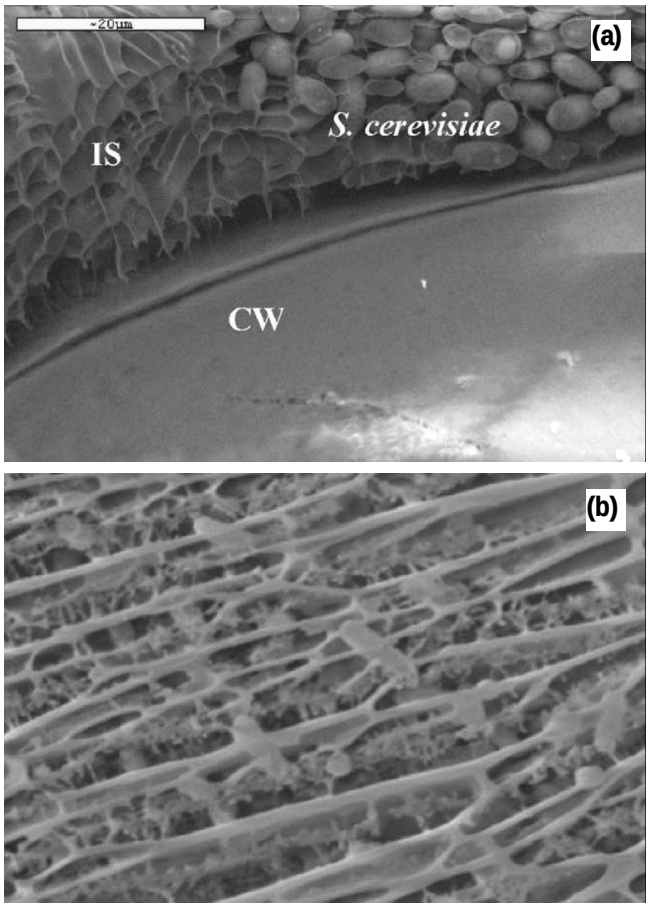

Fig 21: (a) *S. cerevisiae* (magnification: 2000) and (b) *L. casei* (magnification: 7500) in intercellular space of impregnated apple tissue (CW: cell wall; IS: intercellular space full of impregnation liquid with S. cerevisiae cells) (Betrot et al., 2003).