Effects of heat processing on product quality of sous-vide broccoli packs.

by

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Summary

To establish the optimum pasteurisation procedure, the effects of sous-vide heat processing on the pH, ascorbic acid and sensory aspects of broccoli (*Brassica oleracea var. Italica*) were studied.

Broccoli florets were blanched using steam and ammonia(NH_3)-steam, chilled to <2°C in ice water at 0°C, and vacuum packaged. Sous-vide processing proceeded immediately at pasteurisation temperatures 73°, 76°, 79°, 82° and 85°C to a P_{70}^{10} = 100. Directly afterwards, the pH of the processed sous-vide broccoli was measured and percentage L-ascorbic acid loss calculated, by comparing sous-vide broccoli with unprocessed controls from the same broccoli head. Objective colour measurements were made using CIELAB L*, a* and b* co-ordinates. Consumer acceptance was tested using paired preference analysis. The same analyses were repeated after storage at 2° - 4°C for 21 days.

Suitable statistical analyses showed significantly higher ($P<0.05$) pH-values in the case of NH_3-steam-blanched broccoli, with the result that the ascorbic acid retention of NH_3-steam broccoli was significantly lower ($P<0.05$) than that of steam blanched broccoli. Higher pasteurisation temperatures, and resulting shorter heat processing times,

showed a significant reduction ($P<0.05$) in ascorbic acid losses.

NH_3-steam blanched broccoli was significantly ($P<0.05$) greener after pasteurisation than steam-blanched broccoli. Steam blanched broccoli deteriorated further significantly ($P<0.05$) towards a more yellow colour during a 21 day storage period at 2° - 4°C.

Sensory analysis showed a significant consumer preference ($P<0.05$) for the greener NH_3-steam- blanched broccoli but showed a significant preference ($P<0.05$) for the texture of steam blanched broccoli. No significant ($P>0.05$) taste preference was detected for broccoli from either blanching method.

Opsomming

Ten einde die optimum pasteurisasieprosedure vas te stel, is die invloed van sous-vide hitteprosessering op die pH, askorbiensuur en sensoriese aspekte van brokkoli (*Brassica oleracea var. Italica*) bestudeer.

Brokkoli blomme is geblansjeer in stoom en ammoniak(NH_3)-stoom, verkoel tot <2°C in yswater by 0°C, en vakuumverpak. Daar is dadelik met sous-vide prosessering by pasteurisasie temperature 73°, 76°, 79°, 82°C en 85°C, tot 'n P_{70}^{10} = 100 voortgegaan. Direk daarna, is die pH van die geprosesseerde sous-vide brokkoli gemeet en die persentasie L-askorbiensuurverlies bereken, deur sous-vide brokkoli met ongeprosesseerde kontroles, afkomstig van die selfde brokkoli kop, te vergelyk. Objektiewe kleurmetings is uitgevoer deur CIELAB L*, a* en b* koördinate te gebruik. Verbruikersaanvaarding is getoets deur van afgepaarde voorkeuranalise gebruik te maak. Dieselfde analises is herhaal na berging by 2° - 4°C vir 21 dae.

Geskikte statistiese analise het betekenisvolle hoër ($P<0.05$) pH-waardes by NH_3-stoom geblansjeerde brokkoli gewys, met die gevolg dat die askorbiensuurretensie by NH_3-stoom geblansjeerde brokkoli beduidend laer ($P<0.05$) as by stoom geblansjeerde brokkoli was. Hoër pasteurisasie temperature, en derhalwe korter prosesseringstye, het 'n

betekenisvolle kleiner verlies ($P<0.05$) in askorbiensuur getoon.

NH_3-stoom geblansjeerde brokkoli was beduidend ($P<0.05$) groener na pasteurisasie as stoom geblansjeerde brokkoli. Stoom geblansjeerde brokkoli het beduidend ($P<0.05$) verander na meer geel tydens 'n 21 dae bergingstyd by 2° - 4°C.

Sensoriese analise het 'n betekenisvolle verbruiker voorkeur ($P<0.05$) vir die groener NH_3-stoom geblansjeerde brokkoli getoon. Die paneel het egter 'n betekenisvolle voorkeur ($P<0.05$) vir die tekstuur van die stoom geblansjeerde brokkoli getoon. Geen betekenisvolle ($P>0.05$) smaakvoorkeur vir brokkoli van enige van die blansjeringmetodes is waargeneem nie.

Statement

I, Nicolaas Hugo Vlok, hereby declare that the contents of this thesis represents my own work and opinions expressed and recommendations made are my own and not necessarily that of the Cape Technikon.

I also herewith state that this thesis has not previously been submitted for academic examination towards any qualification at any other academic institution

Signed:.....

at.....Cape Town.....

on the 23rd day of .....March.....1998

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CHAPTER 1

LITERATURE REVIEW

"The current trend in the food industry is to reduce the thermal process used for sterilising food products and hence improve the overall quality. For this purpose much research work has been carried out on using processes which reduce the heating time."

(Holdsworth, 1997)

1.1 Introduction

1.1.1 Sous-vide: The current situation.

The sous-vide process of preparing high-quality food was developed in the mid-1970's in France by chef Georges Pralus, and in England by chef Albert Roux, in collaboration with W R Grace Ltd (Schafheitle, 1990). Pralus initially tried to reduce the processing losses in *paté de foie gras*[1] and succeeded in doing so by packing the paté in three plastic layers. The losses were reduced from 40% down to 5% in addition to a more acceptable colour, aroma and taste (Schafheitle, 1987). Since then the flexibility of the method in providing high-quality meals proved to be the main advantage of the process to caterers of all sizes. (Schafheitle, 1990)

[1] Goose liver paté

During the mid 1980's, the process emerged commercially to much publicity given to the excellent sensory quality of the dishes. However, Creed *et al.* (1996) reported that a review of literature up to 1995, showed 70 studies on microbiological safety in comparison to only 30 on nutritional and sensory aspects. Concerns that existed at the time regarding the microbiological safety of sous-vide products, were emphasised by the study and were probably partly responsible for the failing of companies such as Home Rouxl (United Kingdom), Culinary Brands (United States) and several in Canada during the last few years. Martens (1996), however, suggested the following as some possible additional reasons for the failure of these companies:

- *Bad market analysis* - eg: some tried to compete with frozen foods, others overestimated the effectiveness of the local cold chain, ie. residence time and bad temperature control.
- *Technology shortcomings* - eg: Few designers of equipment have a good enough insight into the sous-vide system. Operators of sous-vide kitchens did not have sufficient microbiological background to understand critical control points.
- *Bad communication* - eg: The FDA[2] reacted negatively to sous-vide technology when some independent operators were found to have started sous-vide without the prior consent of the FDA. Small and medium sized operators do not have good relationships with health authorities.

[2] Food and Drug Administration of the United States of America

Multinational companies with large research facilities usually ask for very strict hygiene regulations. In contrast with manufacturers, food service industries such as hotels, airline caterers, cruise operators and railways continued using sous-vide in-house with much less publicity (Creed *et al.* 1996). Smith *et al.* (1990b) predicted that the 1990 sous-vide market in Canada of US$20 million could triple by the year 2000, mainly at the expense of frozen and canned foods.

Much more information on GMP[3] and HACCP[4] specifically for sous-vide products is now available to the prospective producer. Computer aided systems are being developed to assist in the design of safe foods, predictive microbiology and simplification of HACCP procedures (Nicolaï *et al.* 1996). Other systems are being developed to establish the *Just In Time* logistic system in sous-vide processing. In order to simplify the use of some of the computer aided systems, attempts have been made to combine some of the features of different programs. However, very few small or medium sized companies can afford this type of technology (Martens, 1996). New technology in the in-line detection of leaks in sous-vide packs was studied by Dean (1996) and showed a definite move towards attempting to ascertain the quality and integrity of packs and materials. This also serve in addressing in part the microbiological concerns many workers have. This study concerned itself with the effects of a pasteurisation procedure and not by improving the method.

[3] Good Manufacturing Practices
[4] Hazard Analysis, Critical Control Points

1.1.2 Benefits and drawbacks of the sous-vide process.

Sous-vide processing results in a handy pack containing high quality product with superior qualities because it cooked in its own juices which sealed in flavour and aroma (Rhodehamel, 1992; Varoquaux & Nguyen, 1993). Volatiles stay within the package and little nutrients are lost. Vacuum packaging partially removes oxygen which in turn inhibits certain microorganisms and also oxidative and other chemical processes. (Rhodehamel, 1992; Schafheitle, 1990)

It is claimed that the main benefits of this process lies in the extension of shelf-life and both sensory and nutritional eating quality, over ordinary minimally processed foods, but in doing so increase microbiological risk (Crandall *et al.* 1994; Rhodehamel, 1992). The final shelf-life and overall product quality of sous-vide products, are influenced by the following factors which also emphasise the sensitivity of the products to certain conditions (Rhodehamel, 1992):

- Sous-vide products are generally produced with little or no preservatives.
- Sous-vide products receive minimal thermal processing and are therefore not shelf-stable at room temperature, but needs to be refrigerated until needed and then only for a limited time.
- Vacuum packaging provides an anaerobic environment which extends shelf-life by inhibiting normal aerobic spoilage microorganisms. Anaerobic conditions however, provides a

suitable environment for anaerobic food borne pathogens such as *Clostridium botulinum.*

- Mild heat treatments, below normal sterilisation temperatures, together with vacuum conditions may actually select for *Clostridium botulinum* and hence increase the potential for botulism.
- Adequate refrigeration[5] is essential to be maintained in order to prevent outgrowth of *Clostridium botulinum* and the subsequent production of toxin.

Data by Lund (1977) claim that sensitivity of sensory attributes to pasteurisation has been overstated by some authors. However, some products like broccoli and other vegetables are more sensitive in terms of structure than others which raises the potential necessity of specifying different treatments for different products based on relative heat sensitivity and microbiological risk. Church & Parsons (1993) reported the lack of data available regarding the relative effect of sous-vide pasteurisation and conventional cook-chill[6] treatments on nutritional quality.

It became common practice to analyse for heat labile vitamins such as Vitamins B_1, B_2, or C when quantifying changes in nutritional quality of foods during heat processing (Creed, 1995).

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[5] Refrigeration as close to 0°C as possible, without freezing

[6] A process where food is processed under normal conditions, immediately chilled and dispatched to the end user who also keep it refrigerated until needed. Shelf-life is in the order of 5 days maximum. (Schafheitle, 1987)

Contradictory claims as to the vitamin retention as an indication of nutritional quality were recently published. Eriksen *et al.* (1996) reported the advantage of sous-vide processing over MAP[7] and cook-chill processes in terms of vitamin retention, even during cold storage. After 1 day storage the ascorbic acid retention was 80%, 68% and 50%, and after 7 days 72%, 60% and 10% respectively. Walker *et al.* (1996) on the other hand claimed that preliminary studies on Brussels sprouts suggested that no real advantage over simulated optimum cook-serve[8] systems could be established.

Sheard and Church (1992) reported that a lack of uniformity in processing necessitates considerable improvements in process design and control. The use of water as a cooking medium would, in all probability, ensure a more uniform treatment and may offer a possible solution to this problem. Xie & Sheard (1995) reported that the sous-vide process is associated with variations in food uniformity as is the case with sterilisation procedures.

1.2 Broccoli:

1.2.1 Economics

The average annual sales in the Republic of South Africa of fresh broccoli (*Brassica oleraseae* var *Italica*) over the four years 1993 to 1996, totalled on average 1965 tonnes worth R3.75

[7] Modified atmosphere packaging.
[8] A process where food is processed using traditional methods and served immediately.

million per annum (South Africa, 1997). During the period 1994 to 1997, just over 25 000 tonnes were exported fresh or in frozen form (Van Zyl, 1998), having a total value of approximately R47 million in terms of average local market price.

1.2.2 Cultivation

Broccoli requires 60 - 70 days from planting to reach maturity after which the broccoli is harvested (Ronsivalli & Vieira, 1992). It is a good source of Vitamin C (L-ascorbic acid) and is present at levels ranging from approximately 90 to 98 $mg.100g^{-1}$ (Odland & Eheart, 1975; Petersen, 1993).

1.3 The sous-vide process:

The sous-vide process is an extension of the conventional cook-chill[9] process, in the sense that an additional vacuum stage was added to the process (Church & Parsons,1993). This additional stage involves the placing of raw or partly cooked food in an impermeable laminated pouch, which is then evacuated and hermetically sealed after which heat processing of the food, in the bag, follows either for immediate consumption, or to ensure pasteurisation to effect a reasonable shelf-life (Schafheitle, 1990).

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[9] Cook-chill refers to a process whereby food is prepared and immediately refrigerated prior to distribution to the user where the food is stored under refrigeration for a limited time only (maximum shelf-life = ±5 days) before heating and serving.

The basic steps in sous-vide processing is listed in Annexure 1, and involve the following as adapted from Schafheitle (1987):

For in-house use:

1. Prepare raw ingredients, e.g. wash, trim, etc. prior to cooking.
2. Pre-cook material to be processed, e.g. blanch.
3. Place prepared food into high barrier heat resistant plastic bags or pouches.
4. Vacuumise the bag or pouch to remove all residual air and seal bag immediately hermetically before equalisation of the atmospheric pressure has occurred. (Mason *et al.* 1990)
5. Pasteurise the packaged food at a set time and temperature[10] appropriate to the individual characteristics of the vacuum packed food. This is carried out in atmospheric steamers or water baths in catering, and retorts in processing. (Hrdina-Dubsky, 1989)

The product can be served immediately after heat processing is finished.

For retail use:

If however the food is to be distributed and/or stored, the following additional steps are applied:

6. Chill the packs immediately after pasteurisation to 1° - 3°C, within 90 minutes.
7. Label each bag with details regarding the name of the dish, date of production, use-by date, etc.

[10] Previously determined to ensure that proven microbiologically safe levels have been achieved.

8. Store for up to a maximum period of 21 days[11] at 0° - 3°C. (Schafheitle & Light, 1989)

Further adaptations of the process can be seen in Figure 1.1, where Model 1 shows a process found in high-class restaurants where the ingredients is prepared in the normal way, packaged and vacuumised. Instead of immediate pasteurisation following vacuumising, the bags are kept refrigerated until needed and only then pasteurised. If required, a freshly made sauce or other garnish is then added immediately prior to serving.

Model 2 on the other hand shows the work flow of a sous-vide system for food dishes requiring less specialised emphasis. These dishes are usually more economic in range and appeal. Pasteurisation in this case, takes place in the usual way prior to chilled storage. This is the variation to the cook-chill method as mentioned in the Introduction (Schafheitle, 1987).

1.4 Hazard control.

A threat of a major microbiological hazard in the form of growth and toxin production by *Clostridium botulinum* types A, B and E definitely exists (Smith *et al.* 1990a). The principle reasons for this are:

[11] Will differ from product to product and will also depend on initial product quality.

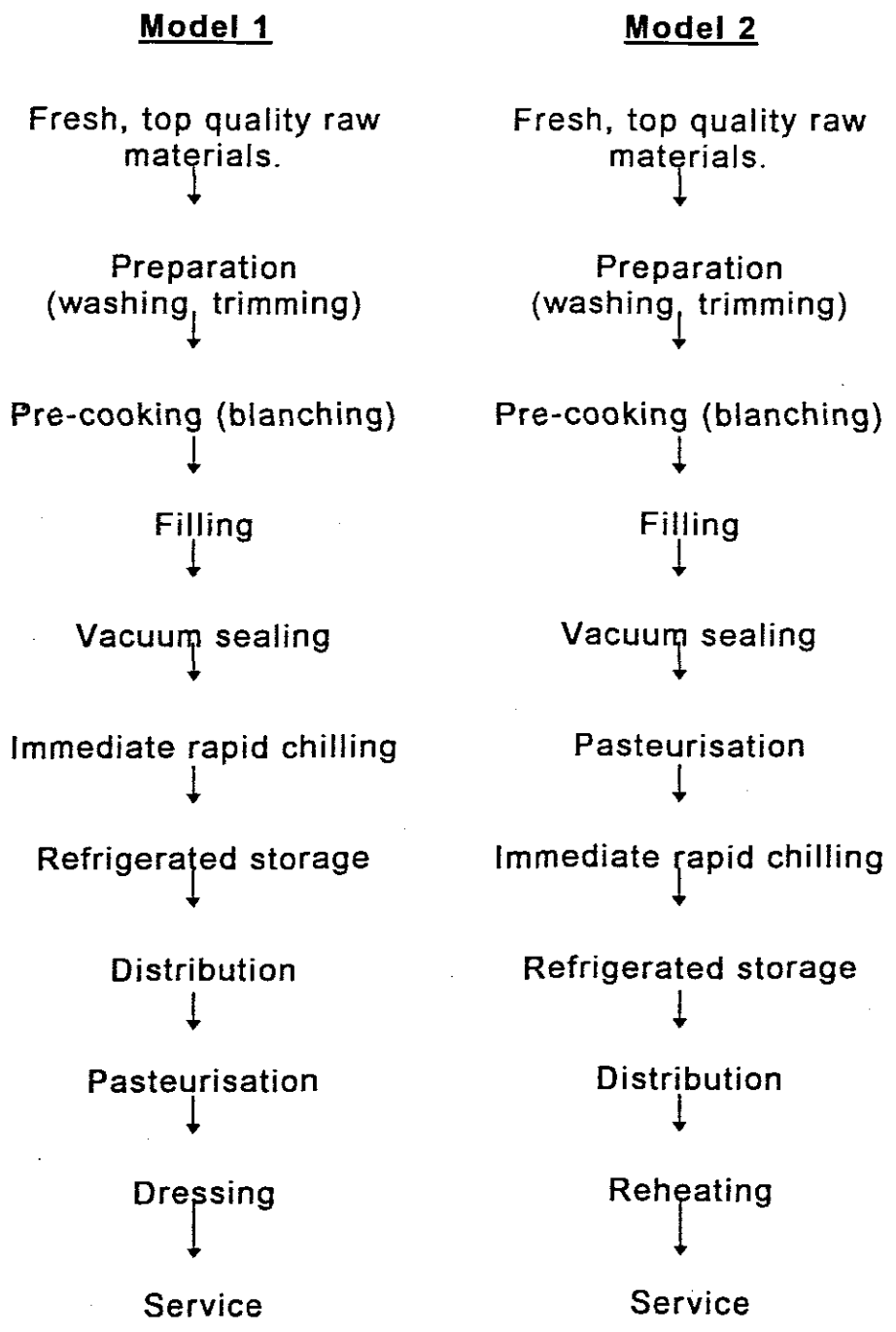


Figure 1.1: Two typical sous-vide workflow models

- the anaerobic packaging conditions in the pack conducive to growth and toxin production by *Clostridium botulinum* in the processed product.
- these spore-formers are able to withstand the mild heat-processing of the sous-vide process,
- the complete or partial destruction of competing non-spore-forming micro-organisms,

1.4.1 Controlling and preventing the hazard.

The development of codes of practice covering extended shelf-life foods are being developed by several organisations (Schellekens, 1996). Various workers have advocated the implementation of HACCP practices in the preparation of food for sous-vide processing (Baird, 1990; Schafheitle, 1990; Smith *et al.* 1990b; Varoquaux & Nguyen, 1993). GMP also should be strictly adhered to as far as sanitation guidelines during preparation are concerned (Rhodehamel, 1992, Schafheitle, 1990). According to Schellekens (1996), France is often serving as the example of regulation for sous-vide products. The debate is ongoing.

1.5 Thermal processing:

1.5.1 Thermal kinetics

Bacteria have a logarithmic order of death when exposed to heat (Potter & Hotchkiss, 1995). A bacterium is said to be dead if it has lost its ability to reproduce (Simpson, 1993). A plot of the

logarithm of the number of surviving microorganisms versus time of exposure gives a straight line, as is shown in Annexure 2. The slope of this curve defines the decimal reduction time (D-value) which is the time required to reduce the microbial load by 1 log cycle.

Complete destruction of a microbial population is theoretically not possible with the logarithmic order of death, hence, thermal destruction time (TDT) is usually defined. This TDT indicate a minimum heating treatment after which no growth occurs. The TDT is dependent on the initial population and therefore represents a certain multiple of D-value which is independent of the initial population.

Stumbo (1973) suggested the following relationship for the mathematical determination of the D-value:

$$D = t / (\log a - \log b)$$

where,

- t = time in minutes of heating at a constant lethal temperature
- a = initial number of viable microbial cells in the population
- b = number of surviving microbial cells in population after time, t.

1.5.2 Unit of Lethality

Sous-vide processing involves less severe heating than sterilisation processes. Instead, a pasteurisation step is introduced to destroy potential non-spore-forming pathogens and normal spoilage flora (Briley, 1992).

The unit chosen for food pasteurisation and sterilisation processes is described by Stumbo (1973) as being 1 minute of heating at a reference temperature - usually 121.1°C. For total process lethality, e.g. sterilisation, the symbol F is used. In the case of sous-vide processing, the symbol P_v is used. P_v indicates the pasteurisation value and is generally determined by Ball's equation (Ghazala & Aucoin, 1996):

$$P_v = \int_o^t 10^{\frac{(T-T_o)}{z}} \, dt$$

where:

P_v = integrated pasteurisation value at the point of slowest heating

t = processing time, minutes

T = temperature at time t, °C

T_o = reference temperature = 70°C

z = z-value of indicator organism, i.e. the slope of the logarithm of the decimal reduction time versus temperature for a specific temperature, °C

1.5.3 Blanching of vegetables.

The process involves heating pre-sliced product in water or steam for a predetermined time and then chilling it as quick as possible at the end of the heating time. Some of the main objectives for blanching vegetables prior to further processing are (Brennan *et al.* 1990):

- to inactivate destructive enzymes
- fix colour
- drive out occluded air for proper evacuation

Blanching is commonly applied to vegetables prior to further processing, such as freezing, sterilisation or pasteurisation, mainly to inactivate harmful enzymes such as catalase and peroxidase enzymes (Dietz & Erdman, 1989; Potter & Hotchkiss, 1995). The effects of these enzymes could lead to adverse effects of colour, texture, flavour and nutritive value.

1.5.4 Effects of heat processes on colour.

Schafheitle (1990) reported that green vegetables tend to lose their green colour and become yellow if they are vacuum cooked without prior blanching and chilling. The loss of green colour, or change in colour rather, is ascribed to the formation of pheophytin from chlorophyll. Pheophytin has a grey-brownish-olive colour. (Berk, 1980; Wong, 1989).

Chlorophyll exists as chlorophyll a and chlorophyll b, differing only on the R- group attached to C-3, with chlorophyll b being the more heat stable (Fennema, 1996). The chlorophyll molecule contains one central atom of magnesium connected to the rest of the molecule by a conjugated bond. This molecule is quite stable in weak alkalis. However, when exposed to even weak acids, the magnesium in the molecule becomes more soluble and is replaced by two hydrogen ions from the acid to form pheophytin (Berk, 1980). When the broccoli is exposed to heat, isomerization takes place by the inversion of the C-10 carboxymethoxy group, to form chlorophyll a' and chlorophyll b' isomers (Fennema, 1996).

Colour changes due to chlorophyll degradation in heat treated vegetable tissue is affected by the prevailing pH in the tissue (Fennema,1996). Green plant tissue is naturally acidic, but these acids does not affect chlorophyll as it is bound to lipoproteins. Chlorophyll is thus protected from effects of these acids. However, during heating, as in blanching or pasteurisation, proteins coagulate and the chlorophyll's become exposed to the effects of the acids which accounts for the colour changes to pheophytin during blanching (Berk, 1980)

Odland & Eheart (1975) stated that water blanching in boiling water gave better colour retention than steam blanching, but then caused more water soluble nutrients to leach out of the product into the water. The colour loss

during steam blanching is due to the presence of volatile acids in the enclosed steam. In addition the vegetable tissue also possess acids and together they cause the pheophytin formation from chlorophyll.

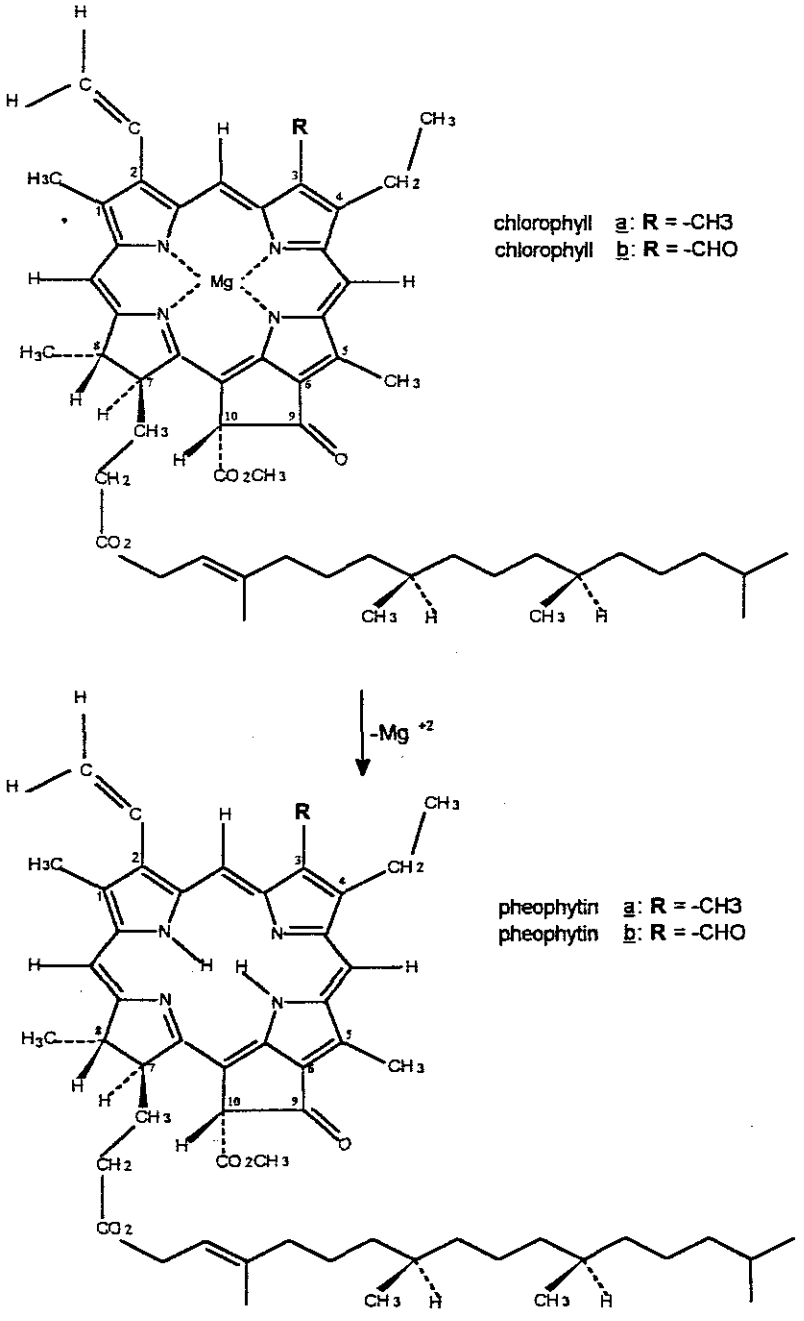


Figure 1.2 Structural formula of chlorophyll showing conversion to pheophytin.

Ronsivalli & Vieira(1992) suggested that by altering the pH of the blanching medium to alkaline, chlorophyllin, which is bright green, is formed instead of pheophytin. They warned, however, that alkaline pH in steam or blanch water may soften vegetable cellulose and cause vegetable texture to become more mushy. This was supported by Potter & Hotchkiss (1995) who further added that water soluble vitamins like ascorbic acid and thiamine may be affected by the higher pH, if blanched in it.

1.5.5 Sous-vide pasteurisation considerations.

Sous-vide products generally receive a 6D process equivalent to 12 minutes at 70°C. This would be sufficient to kill human pathogens such as *Listeria monocytogenes* but not *Clostridium botulinum* spores (Holdsworth, 1997). Sous-vide pasteurisation temperatures are normally below 100°C and processing temperatures above 100°C would therefore not be considered to be true sous-vide processes (Creed, 1995).

A study by Church & Parsons (1993) pointed out that some disagreement still exists about the purpose of the pasteurisation step. Some authors regard sensory quality as a prime concern, and only a minimal process suitable to destroy only the vegetative forms of psychrotolerant anaerobes was recommended. Other authors regarded the reduction of *Clostridium botulinum* spores of prime concern and therefore recommended a more severe heat treatment which meant accepting some compromise of product quality.

A centre temperature of 70°C in order to ensure the destruction of non-spore-forming pathogens in cook-chill foods was suggested by the British Department of Health (Department of Health, Great Britain, 1989). In South Africa no specific pasteurisation procedures are specified as yet, and normal GMP procedures and health regulations apply in the interim (Carolissen-Macay, 1997).

In France, however, treatments are specified by guidelines which base sous-vide processing on *Enterococcus faecalis* as an indicator organism and offer various shelf-life options. A P_{70}^{10} of at least 100 is required for products to attain a shelf-life of between 6 and 21 days after ensuring a SHP[12] temperature of at least 65°C (Church & Parsons, 1993).

1.5.6 Determination of process lethality.

The measurement of the temperature at a selected point in the product being sterilised or pasteurised is paramount to determining process lethality. This is usually done using thermocouples to measure temperature (Holdsworth, 1997).

Thermocouples consist of two very thin wires of different metals, such as copper and constantan[13] , soldered together at one end. At the joint a small electric current is generated

[12] SHP = Slowest Heating Point
[13] Alloy of copper and nickel (usually 45% nickel) having a very high resistance and an extraordinarily low temperature conductivity coefficient.

which changes in voltage when the joint is heated. This phenomenon, called the Seebech-effect, can be measured using a millivolt meter (Eisner, 1988).

The determination of process lethality is broadly divided into General methods, and Formula methods as summarised below (Toledo, 1991):

1.5.6.1 The general method.

Holdsworth (1997), indicated that Bigelow *et al.* described the general method as early as 1920, and integrated the lethal effects of heat treatment by a graphical or numerical integration procedure, based on the time-temperature data obtained during actual commercial processing conditions. Simpson (1993) further suggested that the TDT at the various temperatures during the process be calculated from TDT data obtained using $TDT = 10^{(T_0-T)/z}$.

When the lethal rates (L)[14] is plotted, a curve which integrates the lethal effects of all temperatures during heating and cooling is drawn. The area under the curve is equivalent to the sterilisation value, or as in sub-100°C processes, the pasteurisation value. The cumulative lethality of the process during the entire heating and cooling cycle during pasteurisation is expressed by $P_v = \Sigma L \Delta t$ (Simpson, 1993).

[14] L = Lethal Rate = reciprocal of TDT

1.5.6.2 The formula method.

Various methods to calculate the area under the thermal destruction curve rather than by geometric construction exisits (Holdsworth, 1997). Ball's formula method, according to Simpson (1993), uses the equation:

$$B=f_h \log(j_{ch} I_h / g_c),$$

where,

B = processing time

f_h = heating rate index

j_{ch} = lag factor

I_h = initial temperature difference, $T_{retort}-T_{initial}$

g_c = temperature difference at the end of the cook

Holdsworth (1997) however, states that the following should be kept in mind when using Ball's equation:

- Method applies only to j_c of 1.41
- The curvilinear portion of the cooling curve stops at $j_c = 0{,}141 f_c$
- Method overestimates the F-value when $j_c < 1.41$ and, conversely underestimates the F-value when $j_c > 1.41$
- The cooling phase treatment is less satisfactory than some of those developed by other workers.

where, j_c represents the cooling lag factor, and f_c the cooling rate index.

According to Holdsworth (1997), the formula as described by Stumbo (1973) appears to be more versatile in accounting for thermal effects during cooling, and showed the least errors during a study of five different formula methods investigated. However, both the above methods can easily be applied to pasteurisation systems using a different reference temperatures such as 70°C and a relevant z-value (Simpson, 1993). Stumbo (1973) suggested the following formula for the determination of pasteurisation values:

$$F_i = \log^{-1} \frac{T_x - T_r}{z}$$

where,

F_i = Lethal rate at temperature T_x

T_x = Designated reference temperature

T_r = Retort or processing temperature

z = Reciprocal of the thermal death curve, ie. number of reference temperature degrees required for the thermal death curve to traverse one log cycle.

Eisner (1988) suggested that the F_i be replaced by P_T^z for pasteurisation purposes and that the formula $P_{70}^{10} = D_r (\log N_o - \log N_1)$ be used as a general formula for pasteurisation.

1.6 Quality factors as affected by sous-vide processing.

Packaged foods require sufficient heat for successful thermal processing and hence adequate destruction of microorganisms. It is therefore necessary to establish a time and temperature relationship for achieving this objective (Holdsworth, 1997). The heating effect also cooks the product, give acceptable texture, and destroys active enzymes that otherwise may have caused off flavours, loss of colour and poor texture (Dietz & Erdman, 1989).

1.6.1 pH - value

The pH changes of broccoli after various blanching procedures were described by Odland an Eheart (1975) with steam blanched broccoli having a lower pH than NH_3-steam blanched broccoli due to the alkalinity effects of NH_3 in the steam.

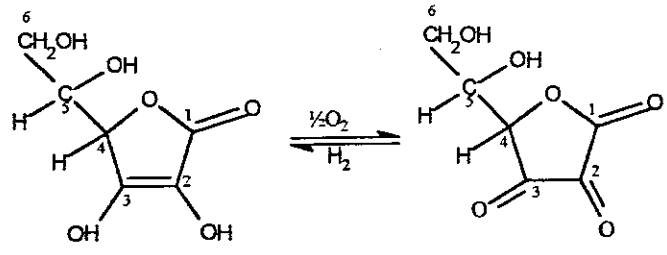
As previously discussed, is chlorophyll degradation related to the pH of vegetable tissue and is the reaction enhanced by acid conditions. The NH_3-steam treatment thus creates an environment less conducive for the formation of pheophytin. This treatment, however, may cause a softening of cellulose and hence vegetable texture (Potter & Hotchkiss, 1995).

1.6.2 Ascorbic acid

Ascorbic acid plays amongst others an important role in tooth formation, healing of broken bones and wounds, and production of certain hormones (Ronsivalli & Vieira, 1992). Ascorbic acid is also important in oxidation-reduction reactions in the body, and is necessary for the normal formation of the protein collagen. Its deficiency could cause conditions such as scurvy, fragile capillary walls and bone joint diseases (Potter & Hotchkiss, 1995).

1.6.2.1 Physical properties of ascorbic acid.

Ascorbic acid in its molecular form does not contain a free carboxyl group even though its name implies the contrary. It is a lactone formed from the loss of water between a carboxyl group on one carbon atom and a hydroxyl group on another (Fox & Cameron, 1970).



L-ascorbic acid Dehydroascorbic acid

Figure 1.3 Structural formulae for L-ascorbic acid and dehydroascorbic acid.

Fennema (1996) explained the oxidation-reduction importance of the two natural vitamers of ascorbic acid, ie. L-ascorbic acid, which is the reduced form, and dehydro-L-ascorbic acid the oxidised form. Both are biologically active in all living tissues. The bioavailability of ascorbic acid in raw broccoli is 20% lower that that in cooked broccoli due to incomplete chewing and/or digestion. However this relatively small difference in bioavailability relative to their cooked forms may have little nutritional significance (Fennema, 1996).

As the most heat labile of the vitamins, ascorbic acid retention or losses is commonly used as a indicator of processing effects on nutrient quality of foods (Petersen, 1993). The losses are mainly due to oxidation, accelerated by heat and light (Dietz & Erdman, 1989). Sous-vide techniques resulted in the greatest retention of all vitamins after heat processing (Eriksen *et al.* 1996). To minimise oxygen content which causes oxidative reactions and microbiological problems, sous-vide products should be vacuumised to at least 98%. Packaging therefore in either laminated plastic pouches or foil-lidded plastic trays is an attempt to achieve just that (Church & Parsons, 1993). The destruction of ascorbic acid in vegetables served after conventional cooking was demonstrated by Walker *et al.* (1996) not to be significantly higher than the same vegetables processed by the cook-chill sous-vide process. However, Petersen (1993) reported that ascorbic acid

retention in broccoli is largely reliant on the vacuum in the sous-vide package. The maximum achievable vacuum should be used. The destruction of ascorbic acid during heat processing may be accelerated by alkaline pH used during blanching (Potter & Hotchkiss, 1995).

1.6.3 Colour

Appearance, and therefore colour, is the most important attribute observed during the judging of food quality (McLaren, 1980; MacDougal, 1984). If this product colour is related to another quality aspect, such as ripeness of fruit or microbial deterioration, it conveys an immediate quality message or perception (MacDougal, 1984). Comprehension of colour systems is required to select a system suitable for measuring colour quality change in products. Clydesdale (1985) reported, however, that a survey conducted indicated that colour is surpassed by freshness as a food choice criterion but ranked higher than taste and texture.

The bright green colour of broccoli is largely due to oil-soluble chlorophyll's which in nature are bound to protein molecules in highly organised complexes (Potter & Hotchkiss, 1995). Methods to stabilise the green pigments of thermally processed vegetables have been described by Clydesdale & Francis, (1968) and Odland & Eheart (1975). The measurement of food colour is meaningful from a quality control perspective by monitoring the effect of processing on colour (Clydesdale, 1985).

1.6.3.1 Objective colour measurement

Colorimetry is simply the means whereby food colour is reported in terms of numerical values (Clydesdale, 1969). McLaren (1980) stated that what the observer perceives about the object, under normal conditions of viewing, is the composition of light entering the observers eye from the object. To classify colour systems, Billmeyer & Saltzman (1981) distinguished between collections of physical colour samples and those not based on actual samples. Systems for measuring colour are concerned with processing the data mathematically in order to make meaningful deductions concerning the colour of an object.

The Munsell system.

This system is representative of the colour systems using physical colour samples. An advantage of the system is its conformance to equal visual perception. The system is also both a collection of samples painted to represent equal intervals of visual perception between adjacent samples, and a system describing all possible colours is described of its three co-ordinates, which are defined as follows (Billmeyer & Saltzman, 1981):

Value: that quality of colour which is described by the words light, dark, etc. relating the colour to a grey of similar lightness.

Hue: that quality of colour which is described by the words red, yellow, green, etc.

Chroma: that quality which describes the extent to which a colour differs from a grey from the same value.

Clydesdale (1969) described the system as a colour model where the vertical central axis is representing the value. Hue is represented on the horizontal circumference of the colour solid around the value axis at the centre. Chroma is represented by distance units from the central axis. Figure 1.4 shows the Munsell colour solid and co-ordinates (Billmeyer & Saltzman, 1981).

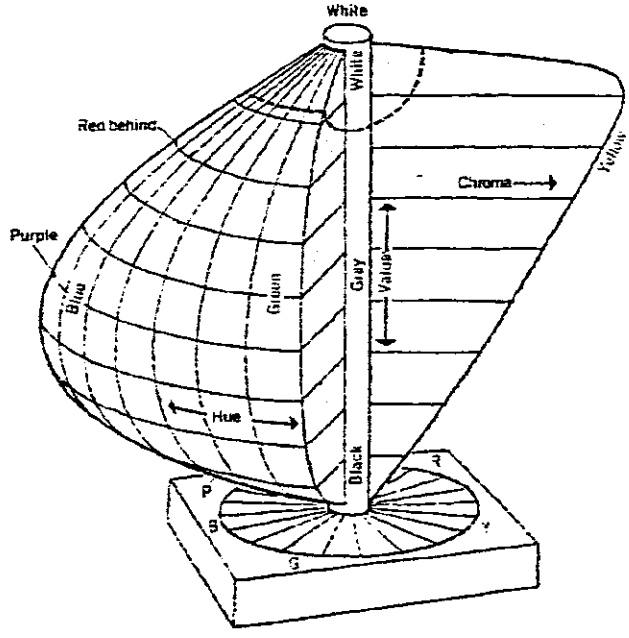


Figure 1.4: Munsell Colour Solid showing orientation of Value, Hue and Chroma.

The CIE system.

A method for quantifying the surface colour of food in terms of numerical co-ordinates was introduced by the CIE[15] in 1931. The co-ordinates, X, Y, and Z, replaced the primary colours, red (R), green (G), and blue (B) previously used (Clydesdale, 1969). These imaginary primaries gave an all-positive set of values instead of the sometimes negative matching required by the 'real' primaries R, G, and B (MacDougall, 1984, Billmeyer & Saltzman, 1981). These primaries were also selected so that a relatively large range of colours in the yellow-red region could be matched with only two primaries, and the intensity or lightness is specified by the Y primary alone (Clydesdale, 1969). In obtaining two-dimensional maps of colours, chromaticity co-ordinates are calculated. These co-ordinates describe the qualities of a colour in addition to its luminance factor. In the CIE system, the chromaticity co-ordinates *x, y,* and *z* are obtained by the following equations (Clydesdale, 1969; Billmeyer & Saltzman, 1981):

$$x = X(X+Y+Z),\ y = Y(X+Y+Z),\ \text{and}\ z = Z(X+Y+Z)$$

Colour can be plotted on a chromaticity diagram using x and y which have been the preferred choice since 1931 (McLaren, 1980). To describe the colour one of the tristimulus values, usually Y, must also be specified (Billmeyer & Saltzmann, 1981).

[15] Commission Internationale de l'Eclairage

The Hunter colour system.

The Hunter (L, a, b) system is represented by the Hunter colour solid and is a non-linear transformation of the 1931 CIE X, Y, Z system. This is typical of the opponent type colour scales (Billmeyer & Saltzman, 1981). Figure 1.5 shows the axis, designated as L, a, and b, of the colour solid (Clydesdale, 1969). The method uses numerical co-ordinates to locate individual colours in a "colour solid" based on uniform visual colour spacing.

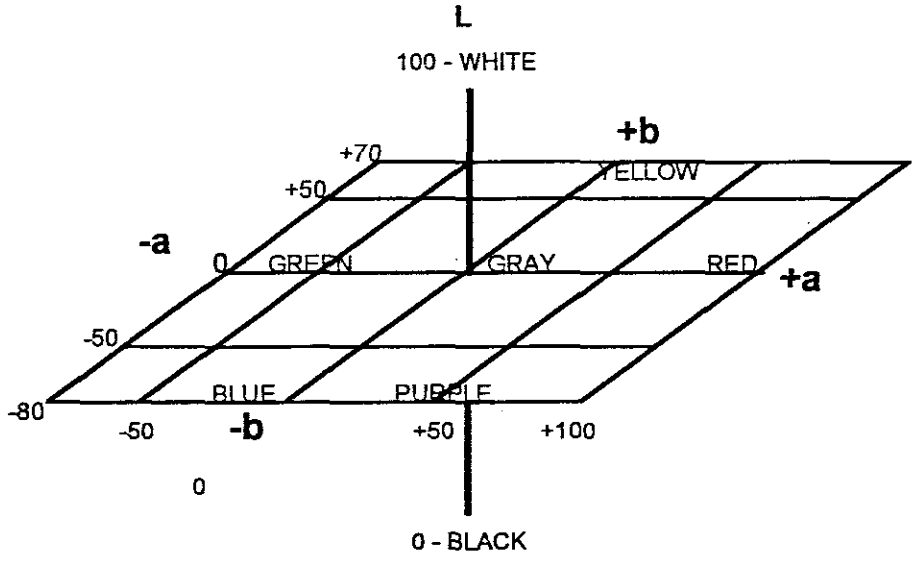


Figure 1.5: **Hunter L, a, b colour solid - opponent type colour scale.**

The CIELAB colour system.

CIE proposed in 1976 the CIE L*, a*, b* colour space which translates the X,Y,Z co-ordinates of the 1931 tristimulus values into L*,a*,b* values, which also forms the axes of a

three dimensional colour space (McLaren, 1980). The L* a*, b* values translation is a non-linear transformation of the 1931 X, Y, Z values, in order to approximate the Munsell system. The values are defined by the following equations (Billmeyer & Saltzman, 1981; MacDougall 1984):

$$L^* = 116(\frac{Y}{Y_n})^{1/3} - 16$$

$$a^* = 500[(\frac{X}{X_n})^{1/3} - (\frac{Y}{Y_n})^{1/3}]$$

$$b^* = 200[(\frac{Y}{Y_n})^{1/3} - (\frac{Z}{Z_n})^{1/3}]$$

were,

X_n, Y_n and Z_n are the tristimulus values of the white reference. The values $\frac{X}{X_n}$, $\frac{Y}{Y_n}$, and $\frac{Z}{Z_n}$ must all be greater than 0.01 (Billmeyer & Saltzman, 1981).

1.6.3.2 Quantifying colour differences.

The conversion of the Cartesian co-ordinates, L*, a*, b*, into the cylindrical co-ordinates L*, C* and H°, quantify the variables of the well known Munsell colour space (McLaren, 1980). The co-ordinates are defined by the following (McGuire, 1992):

L* (Munsell Value) is measured on a 0 (black) - 100 (white) scale.

C* (Munsell Chroma) is calculated by $(a^{*2} + b^{*2})^{0.5}$, and indicates the colour saturation.

H° (Hue-angle) is the angle between the hypotenuse and 0° and the a* axis, and is calculated by $\tan^{-1}$ (b*/a*). A colour change from green to yellow is indicated by a decrease in the hue angle. (Berrang *et al.* 1990). McGuire (1992) suggested the use of both the hue angle (H°) and the chroma (C*), in order to quantify the effects of heat treatment on the colour of foods.

Odland & Eheart (1975) advocated the use of a/b ratios[16], based on Hunter L, a, b co-ordinates, to quantify the conversion of chlorophyll's to pheophytin. The use of a/b was also suggested by Clydesdale (1985) as an analytical tool to simplify the evaluation of greenness to a single value. Clydesdale (1985) also mentioned the calculation of hue angle and chroma using tristimulus values from the Hunter colour solid.

Being able to measure colour difference when comparing a production sample with a standard is desirable in monitoring production uniformity (McLaren, 1980). Extensive research have produced a colour difference equation, CIELAB, which is defined by (Billmeyer & Saltzman, 1981; McLaren, 1980):

$$\Delta E_{(CIELAB)} = [(\Delta L^*)^2 - (\Delta a^*)^2 - (\Delta b^*)^2]^{0.5}$$

[16] Defined as Gardner Values by Odland & Eheart (1975).

If required and if relevant, any colour difference can now be split up into components correlating with hue, value and chroma using the following formulae (McLaren, 1980):

$$\Delta H^* = [(\Delta E)^2 - (\Delta L^*)^2 - (\Delta C^*)^2]^{0.5}$$

$$\Delta L^* = L^*_{sample} - L^*_{standard}$$

$$\Delta C^* = C^*_{sample} - C^*_{standard}$$

The calculated value of each component is expressed in CIELAB units.The ease with which tristimulus values can be converted into single values made the CIELAB system very useful in the food industry. Another colour space, CIELUV, was defined by CIE in 1976 but is intended for industries involved with additive mixing, e.g. lighting and television (McLaren, 1980).

As a result of the above findings, the use of CIELAB co-ordinates was considered appropriate for the purposes of this study and the same formulae, as proposed by Clydesdale (1985), were used for calculating hue angle (H°), chroma (C*) and a*/b* ratio.

1.7 Problem statement

Although the influences of sous-vide processing on vitamin retention and sensory quality have been studied, work still needs to be done on standardising the pasteurisation process to ensure constant product quality and nutrient retention (Church & Parsons, 1993; Petersen, 1993). In

order to produce high quality sous-vide broccoli, and to provide definite production standards, further investigation into the effects of the heat processes on sensory and nutritional aspects was considered necessary.

The aim of this study was thus, to investigate the effects of steam blanching, NH_3-steam-blanching and pasteurisation temperature on sous-vide broccoli in terms of:

- nutritional quality changes by measuring ascorbic acid losses,
- pH changes,
- colour changes through instrumental analysis,
- texture and sensory acceptability using a consumer taste panel,
- the feasibility of standardising the pasteurisation parameters of sous-vide broccoli and in the process optimise sensory characteristics by using sound material and scientific methods of processing, without losing pasteurisation integrity.

CHAPTER 2

MATERIALS AND METHODS

2.1 Raw Materials:

2.1.1 Broccoli.

Broccoli heads (cv 'Viking'), ± 25 per experimental trial, were cut during April - July 1997 from adjacent plants of the same maturity, 6 - 8 weeks, on the afternoon prior to each trial. The heads originated from the same field on the farm Bonne Esperance in the Devon Valley area in the Stellenbosch district (Western Cape Province, South Africa), and were immediately transported in a plastic crate to the processing facility and stored overnight at 2° - 4°C in a refrigerated room.

2.1.2 Packaging material.

High barrier non-shrinking pouches (Cryovac) were obtained from Darex Ltd. (W R Grace, SA). The film was made-up of a 15μ bi-axially orientated nylon layer, in association with a 60μ polyethelene heat sealable layer. The oxygen transmission rate was 15 - 20 $ml/m^2/24$ hours, and the water vapour permeability $7g/m^2/24$ hours. The pouches were made to a convenient size, ie. 200 x 300 mm, to facilitate reasonable flexibility especially during the fitting of packing glands in the pouch wall to allow the insertion of heat penetration thermocouples into the broccoli florets.

2.2 Equipment:

2.2.1 Blanching.

A steam cabinet, 46 cm wide, 76 cm deep and 62 cm high, having an open topped 60 litre water reservoir, in the bottom of the cabinet, was used. The reservoir was equipped with a ball valve water level control assembly and a steam coil. Before blanching commenced, steam at 600 kPa was passed through the steam coil, which served as a heat exchanger, causing the water to boil. The cabinet was equipped with a door able to be bolted closed. A removable stainless steel tray perforated with holes 12 mm in diameter and spaced 32 mm apart fitted inside the cabinet, 315 mm above the surface of the boiling water, and served to hold the broccoli florets to be blanched.

2.2.2 Vacuum packing.

Vacuum packing was carried out in a Multivac Model AGW 1998[1] equipped with gas flushing capabilities and an extra deep chamber suitable for bulk processing. It had the benefit of an observation window so that effects brought about by changes in atmospheric pressure could be observed. The pouches were sealed at the maximum vacuum obtainable, as indicated on the manometer of the vacuum sealing machine. This was tested to be *ca* -99 kPa. Pouches, equipped with a packing gland for a thermocouple, were sealed so that the position of the floret stem as opposed to the packing gland after vacuum sealing,

[1] Supplied by Geiger and Klotzbücher, Cape Town. South Africa.

facilitated easy insertion of the thermocouple without stressing the material. (See Section 2.2.5 and Figure 2.2)

2.2.3 Pasteurisation in retort.

A vertical retort shell (F), having a diameter of 40 cm and a capacity of 100 litres, was modified to recirculate water at a velocity of $\pm 24\ell.min^{-1}$, by adding an outside pump (E) connected to pipes with the suction end at the bottom and the delivery end at the top of the retort shell. Water in the retort-shell was heated by a horizontal steam-coil (D) in the bottom of the shell below a perforated support plate.

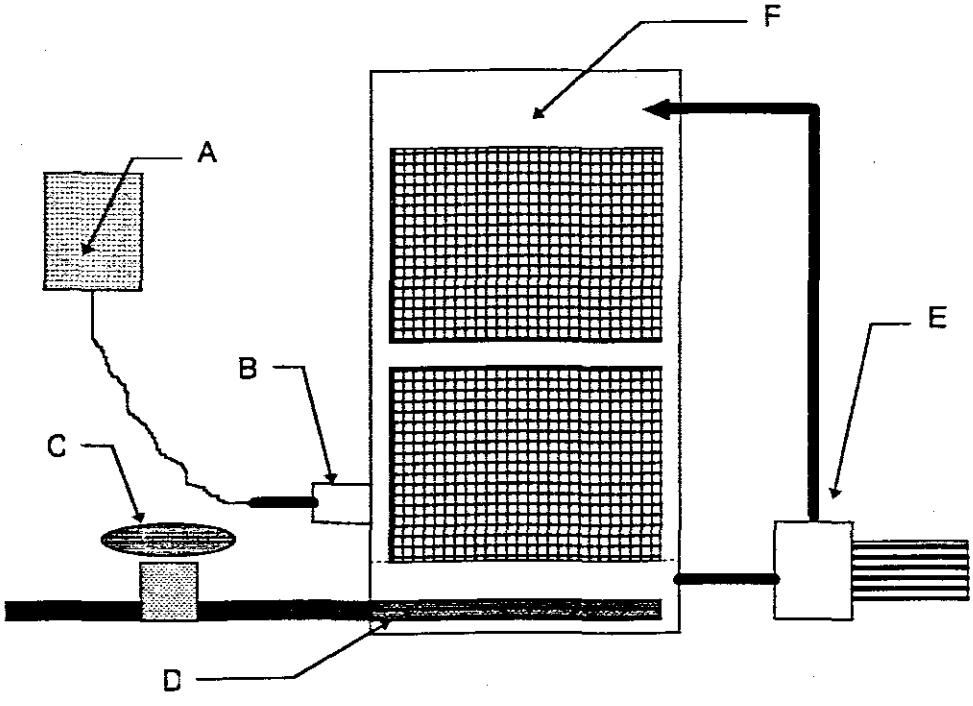


Figure 2.1 Retort-shell with baskets and recirculating pump

A = Controller, B = PT100 Resistance temperature probe, C = Steam valve, D = Steam coil, E = Recirculating pump, F = Retort-shell with two baskets.

Temperature was thermostatically controlled through a pneumatic steam-valve[2] fitted with a pneumatic valve positioner[3] (C). The signal for the opening and closing of the steam-valve was received from a electronic controller[4] (A) coupled to a PT 100[5] resistance temperature probe (B) located in the side of the retort shell.

The temperature was controlled for the various runs at processing temperatures 73°, 76°, 79°, 82° and 85°C. The filled sous-vide pouches were placed in stainless steel wire baskets equipped with stainless steel mesh covers to prevent pouches from floating around or obstructing flow of water. The baskets were placed in the retort shell filled with water circulating at the required temperature. Once the required P_{70}^{10}-value was reached, the baskets were removed and immediately transferred to a cooling tank with water at 1° - 3°C.

2.2.4 Cooling tank.

A refrigerated Japy[6] tank of 500 litre capacity, equipped with a stirrer was used. The water in the tank was kept constant at 1° - 3 °C. The water level (±40cm) in the tank was kept just high enough to cover the retort baskets when placed in it for cooling.

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[2] H D Baumann Model 3224588 supplied by Negretti & Zambra Instrumentation and Control, Cape Town. South Africa.
[3] Honeywell Series 2750 supplied by Negretti & Zambra Instrumentation and Control, Cape Town. South Africa.
[4] Fuji E Z series - Type PYZ 4. Supplied by Negretti & Zambra Instrumentation and Control, Cape Town. South Africa.
[5] Supplied by Negretti & Zambra Instrumentation and Control, Cape Town. South Africa.
[6] Supplied by Anderson Engineering, Pietermaritzburg. South Africa.

2.2.5 Heat penetration measurement.

Control sample pouches for heat penetration measurements of each batch of broccoli were provided with packing glands to facilitate the fitting of thermocouples for heat penetration measurement. The internal temperature SHP[7] of a single broccoli floret in each of at least two packages was monitored throughout the entire heating and cooling process using Ellab Model SSA-12050-G700-TS[8] copper/constantan Type T thermocouples. The thermocouple was passed through an Ellab Model GTK-21009-C000[8] polyoxymethylene compounded packing gland fitted to the pouch wall and then into the broccoli floret.

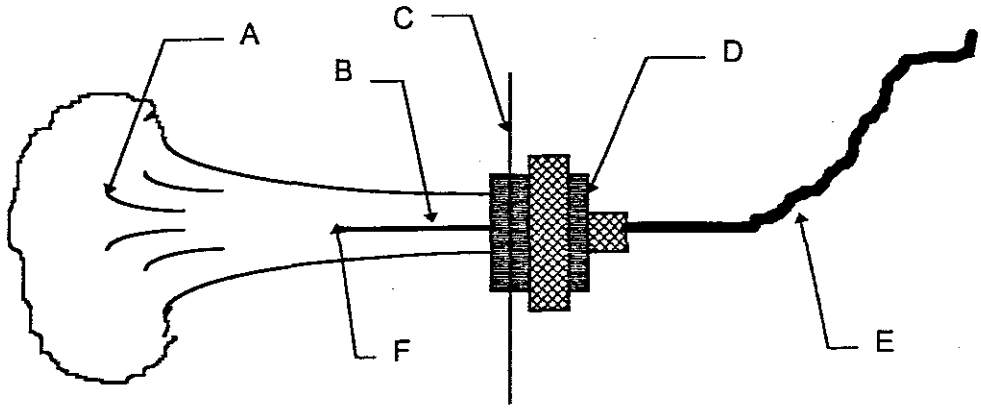

Figure 2.2 Thermocouple and packing gland assembly.

A = Broccoli floret, B = Thermocouple, C = Laminated vacuum bag wall, D = Packing gland assembly, E = Thermocouple cable to data logger, F = Temperature measuring point.

A section of Tesa Multikrafttape[8], able to withstand the processing conditions and mechanical strains, was adhered to

[7] Slowest Heating Point
[8] Supplied by Medical Distributors, Randjespark, Midrand. South Africa.

the outside of the pouch over the area where the hole for the packing gland was to be made. Vacuum integrity was ensured throughout the processing procedure by utilising a silicone septum in the packing gland. The thermocouple needle was placed in the centre of the 25 - 35 mm stalk section of the broccoli floret, so that the sensing point (F) was in the thickest part of the floret stalk.

A Hewlett Packard 3852A data logger[9] fitted with a HP 44708A relay multiplexer[9] was used to constantly log the SHP temperature of the reference pouches equipped with thermocouples. The data were automatically read via a PC and stored in a Microsoft Excel V5.0 spreadsheet. A graph was generated throughout the process to show the temperatures and other calculated values over real time (X-axis). The graph indicated the following:

- Temperature of retort. (Y_1 -axis)
- Temperature of SHP of at least two samples equipped with thermocouples as explained above. (Y_1 -axis)
- Accumulative P_{70}^{10}-value. (Y_2 -axis)
- Processing time. (X-axis)

Upon reaching the required P_{70}^{10}-value, as indicated on the graph, the retort basket containing the pouches were removed from the retort and transferred to the cooling tank.

[9] Supplied by Hi-Performance Systems (Pty) Ltd, Johannesburg, South Africa.

2.3 Sample preparations.

2.3.1 Broccoli cutting and trimming.

Each head of broccoli was separated into individual florets. The florets were selected and trimmed to obtain an average diameter of 50 - 60 mm across, an overall length of 90 - 110 mm, and an average stalk diameter of 15 mm, to enhance uniform thermal effects and measurements. Samples for analysis were prepared so that triplicates from the same head of broccoli were representative of both blanching procedures as well as the full range of pasteurisation temperatures. In addition to the set analysed immediately, samples from the same population were cold stored for 21 days at 2 - 4°C prior to analysis

2.3.2 Blanching.

Two versions of steam blanching were employed, ie.

Steam blanching, and *Ammonia steam-blanching (NH_3-steam-blanching).*

The florets were placed on the perforated stainless steel tray and placed in the cabinet for 3.25 minutes, previously determined as discussed in section 2.6.1, using the method described by Lange (1983). The tray was removed from the cabinet, having a saturated steam atmosphere at 98° - 100°C developed from the water boiling in the reservoir, and immediately dipped in ice water at 0°C. Once cooled to <2°C, the florets were removed with a stainless steel sieve from the water, allowed to drain for 30 seconds while shaking gently to remove excess water, and vacuum packaged immediately.

For NH_3-steam-blanching, 2g.dm^{-3} of NH_4HCO_3 (Odland & Eheart, 1975), was mixed into the boiling water reservoir of the blanch cabinet. A period of 3 minutes of boiling with the door open was allowed before the trays containing the broccoli was blanched. After each blanching run with NH_4HCO_3, the reservoir was drained and thoroughly cleaned to ensure no residue remained that may influence subsequent blanching.

2.4 Pasteurisation.

Where applicable, thermocouples were inserted through the previously fitted packing gland into the pouches, and the thermocouple cables securely fastened. The sample pouches were placed in one of the retort baskets in such a way as to allow free flowing of water between pouches. A stainless steel mesh cover kept the pouches submerged and in place throughout the pasteurisation step.

The basket with the pouches was lowered into the preheated water and the computer program monitoring of the heat penetration was started. Once a P_{70}^{10}-value of 100 was reached, the basket was removed from the retort and immediately transferred to the cooling tank at 1°- 3°C.

2.5 Storage.

Pouches destined for immediate analysis were kept in the cooling tank until needed, while an identical set was transferred to a refrigerator, to be stored at a temperature of 2° - 4°C and further analyses after 21 days, once the SHP has reached <4°C.

2.6 Analytical Methods.

2.6.1 Enzyme activity: Blanching time

Peroxidase activity after blanching was analysed for according to Lange (1983). Blanched tissue, ±5 g from the thickest part of the floret stem, was exposed to a mixture of 5 ml water, 1 ml of a 1% Guiacol (1-hydroxy 2-methoxybenzole) in 95% ethanol solution, and I ml of 0.5% hydrogen peroxide solution. A red-brown discoloration within 2 - 5 minutes of the exposed tissue indicated peroxidase activity and hence an indication of insufficient blanching. The blanching time was considered to be the minimum time required after closing the door of the blanching cabinet to get a negative peroxidase activity.

2.6.2 Ascorbic acid content.

L-ascorbic acid content in the broccoli was determined after processing and again after cold storage for 21 days at 2° - 4°C. A method was used where 2,6-Dichlorophenol-indophenol was reduced to its colourless form, by L-ascorbic acid (Horwitz, 1980). The method was adapted, as described by Buckley

(1987), by first macerating the broccoli samples under oxalic acid using a Kinematica CH6010 Blender[10], followed by centrifugation for 15 minutes at 4000 rpm. using a MSE Minor centrifuge[11]. The supernatant was poured off for use in the analysis.

2.6.2.1 Calculation of ascorbic acid losses.

The ascorbic acid losses were calculated by comparing the L-ascorbic acid content in the samples to that of the fresh[12] control sample. The comparison between heat treatments immediately after pasteurisation, and again after 21 day's storage at 2° - 4°C, was done on triplicate samples originating from the same head of broccoli. Ascorbic acid losses were calculated as percentage L-ascorbic acid losses.

The following procedure was followed:

L-ascorbic acid content of fresh broccoli = X mg.100g^{-1}

L-ascorbic acid content of processed broccoli = Y mg.100g^{-1}

L-ascorbic acid losses = $X - Y$ mg.100g^{-1} = Z

% L-ascorbic acid Losses = $\frac{Z}{X}$ x 100

[10] Supplied by Scientific Associates, Cape Town, South Africa.
[11] Supplied by Labotec, Cape Town, South Africa.
[12] Unblanched and unpasteurised

2.6.3 pH measurement.

The same pouches used for ascorbic acid analysis were used for pH measurements. A Beckman Model pHI 100 pH meter equipped with a ISFET penetration probe[13] was used. The pH meter was calibrated directly prior to measuring. pH measurements were made by pushing the electrode into the thickest part of a floret after carefully opening the pouch. The pouches were allowed to reach ambient temperature before triplicate pH-readings per pouch were made.

2.6.4 Colour measurement.

A Byk-Gardner Handicolor Colorimeter[14] was used for objective colour measurement of the processed broccoli. The colorimeter was calibrated using the black[15] and white[16] standard tiles. Performance of the colorimeter was verified using a green[17] standard tile. For measurement to be repeatable, florets were cut as was discussed under 2.3, and the floret heads selected such that they were >5cm across to cover the measuring aperture on the colorimeter, excluding external light successfully from entering. Three measurements were made on each sample after turning the floret one third, and the mean[18] of the three measurements[19] noted. Measurements were repeated for each of the three replicates respectively. These values were used to calculate the following:

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[13] Supplied by Beckman Instruments, Cape Town, South Africa.
[14] Supplied by Premier Technologies, Cape Town. South Africa.
[15] S/N 92005315, L*=-0.03, a*=-0.13, b*=0.30
[16] L*=96.45, a*=-0.81, b*0.12
[17] L*=75.65, a*=-10.64, b*=11.56
[18] The colorimeter calculated the mean of each triplicate reading on a floret
[19] L*, a* and b* reading after turning the floret by one third.

- Hue angle (H°) using the equation, $\tan^{-1}b^*/a^*$
- Chroma (Saturation index) given by, $C^* = (a^{*2}+b^{*2})^{0.5}$
- a*/b* Ratio

2.6.5 Sensory analysis

A 30 member consumer panel was used to test quality perception in terms of colour, texture and taste acceptability using paired preference analysis (Heymann, 1995). The panellists were selected to be representative of the middle income group and consisted of 16 females and 14 males. All members were in the 35 - 45 age group.

Tasting took place at 14h00 on three successive days in a demonstration kitchen at the Cape Technikon. To simulate a household atmosphere, no special lighting was provided. Light in the kitchen was by a combination of neon tubes from above, and natural light from the left of the panellists. The panellists were presented with samples on a white tray, representing each of the two blanching methods. Members received samples coded differently and in different sequences. Only one of the sensory aspects was evaluated at a time by instructing the panellists to indicate their preference first for the required aspect, eg. colour preference, while ignoring taste or texture. The same was then repeated evaluating the other two aspects, again ignoring the remaining aspects.

2.6.6 Statistical analysis.

Ascorbic acid, pH and colour analysis results were subjected to statistical analysis using Microsoft Excel V5.0, and Statistica V5.1[20]. Differences between treatments[21] were statistically determined using Analysis of Variance.

Rates of change and effects of temperatures were assessed by regression analysis using the least squares method.

For sensory analysis, two-tailed paired preference analysis was used using Roessler tables (Heymann, 1995).

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[20] Statsoft Inc., 2325 East Street, Tulsa, Oklahoma. USA. 74104.

[21] Differences were calculated between blanching procedures at the various pasteurisation temperatures as well as differences in the effects of temperatures.

CHAPTER 3

RESULTS

3.1 Blanching time: Enzyme activity

Table 3.1 shows the average blanching times determined when establishing the *minimum* time necessary to achieve adequate negative peroxidase activity after blanching.

Table 3.1: Peroxidase activity of broccoli florets after blanching time intervals.

	Minutes after closing cabinet door				
Blanching time	2.50	2.75	3.00	3.25	3.50
Peroxidase activity[a]	+++	+++	++ -	- - -	- - -

[a] Three separate florets analysed per time interval

After 3 minutes from closing the cabinet door until opening it and cooling the broccoli, two of the three replicates gave positive results. Therefore, blanching time was determined to *be not less than 3.25 minutes*[1] *for the florets as prepared* under 2.3.

[1] The next blanching interval, i.e. +15 seconds.

3.2 pH changes.

The pH of steam blanched broccoli did not change significantly ($P>0.05$) during the 21 day storage period at 2° - 4°C (Fig.3.1/Annexure 3.3).

NH_3-steam-blanched broccoli, on the other hand, showed a *significant ($P<0.05$) difference in pH between Day 0 and Day* 21 (Fig. 3.1/Annexure 3.4). A significant ($P<0.05$) increase in pH with an increase in pasteurisation temperatures was detected only in NH_3-steam-blanched broccoli of Day 0 (Fig.3.1/Annexure 3.7).

No significant effects ($P>0.05$) of pasteurisation temperatures on the pH of steam blanched broccoli were observed (Fig. 3.1/Annexure 3.5 & 3.6). The pH of NH_3-steam-blanched sous-vide broccoli was significantly higher ($P<0.001$) compared to steam blanched broccoli on both Day 0 and Day 21.

3.3 Ascorbic acid losses (%).

Regression analysis showed significant reduction ($P<0.05$) in percentage ascorbic acid losses as pasteurisation temperature increased for broccoli from both blanching procedures and both Day's 0 and 21 (Fig. 3.2/Annexures 4.5 - 4.8).

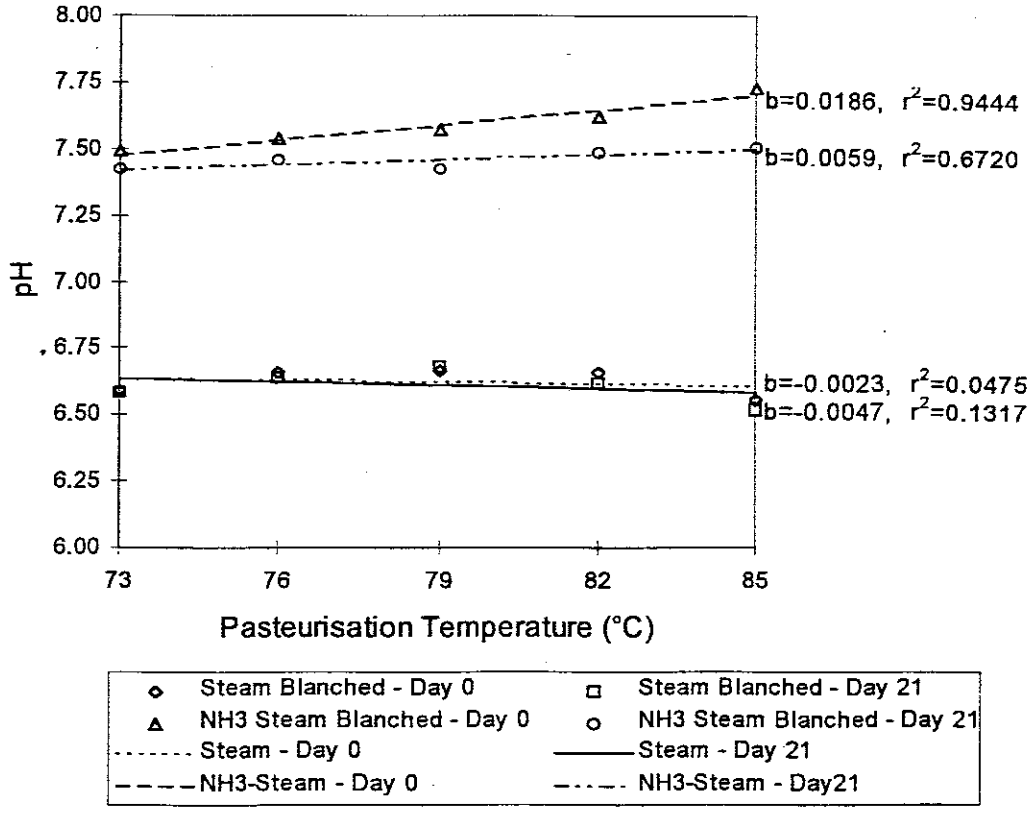

Figure 3.1 **Regression functions that best fit mean[a] pH measurements of broccoli subjected to five heat treatments[b] and two blanching methods[c] at two storage periods[d].**

[a] Triplicate analysis.
[b] Pasteurisation temperatures: 73°, 76°, 79°, 82° and 85°C.
[c] Steam blanching and NH_3-steam blanching.
[d] Directly after sous-vide processing (Day 0) and after being stored for 21 days at 2° - 4°C (Day 21).

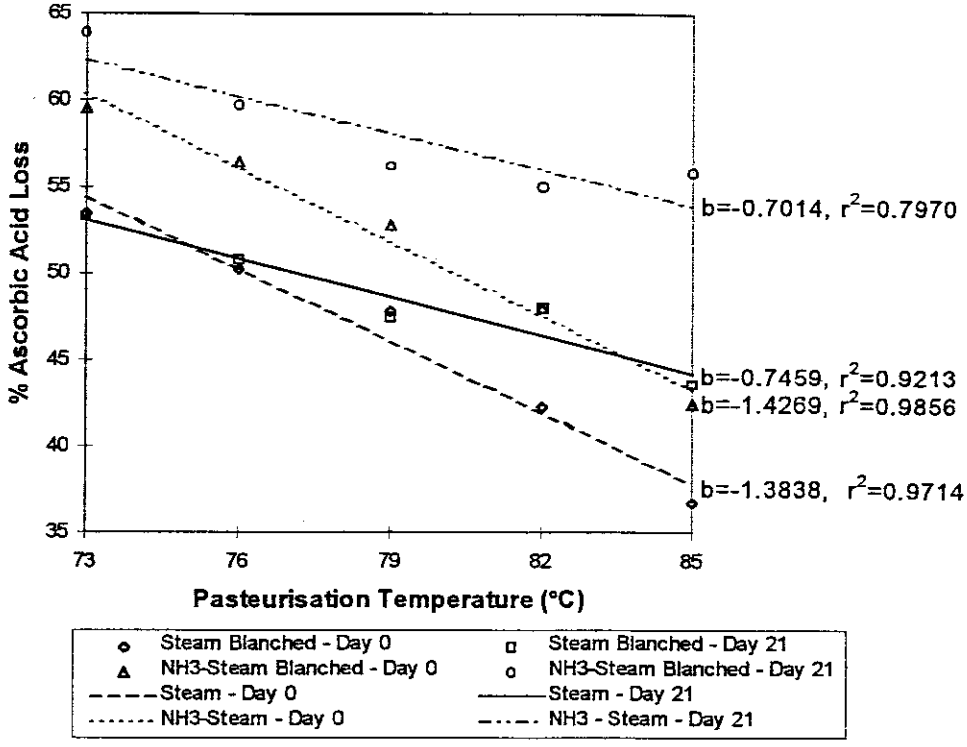

Figure 3.2 **Regression functions best fitting mean[a] percentage ascorbic acid losses in broccoli subjected to five heat treatments[b] and two blanching methods[c] at two storage periods[d].**

[a] Triplicate analysis
[b] Pasteurisation temperatures: 73°, 76°, 79°, 82° and 85°C.
[c] Steam blanching and NH_3-steam blanching.
[d] Directly after sous-vide processing (Day 0) and after being stored for 21 days at 2° - 4°C (Day 21).

The change in the rate of percentage ascorbic acid loss with an increase in pasteurisation temperature for both blanching procedures was less pronounced for samples from Day 21 (Fig.3.2). The ascorbic acid losses of steam blanched broccoli were significantly ($P<0.05$) lower than that of NH_3-steam-blanched broccoli for both Day's 0 and 21 (Fig. 3.2/Annexure 4.1& 4.2). No significant differences ($P>0.05$) in ascorbic acid losses for either steam blanched or NH_3-steam-blanched broccoli were detected between Day 0 and Day 21 (Fig 3.2/Annexure 4.3). The effects of sous-vide processing to $P_{70}^{10}=100$ at 73°, 76°, 79°, 82° and 85°C on the degree of ascorbic acid loss for broccoli steam and NH_3-steam-blanched, are shown in Figure 3.2.

3.4 Colour changes.

3.4.1 a*/b* Ratio

Colour differences, as represented by the a*/b* ratio, of sous-vide broccoli directly after pasteurisation and again after 21 days cold storage at 2° - 4°C are shown in Figure 3.3. Significant losses in greenness ($P<0.05$), on both Day 0 and Day 21, were observed for steam blanched sous-vide broccoli when compared to NH_3-steam-blanched broccoli (Fig. 3.3/Annexure 5.1 & 5.2). The a*/b* of steam blanched broccoli further deteriorated significantly ($P<0.05$) towards yellow-red after 21 days stored at 2° - 4°C (Fig. 3.3/Annexure 5.3).

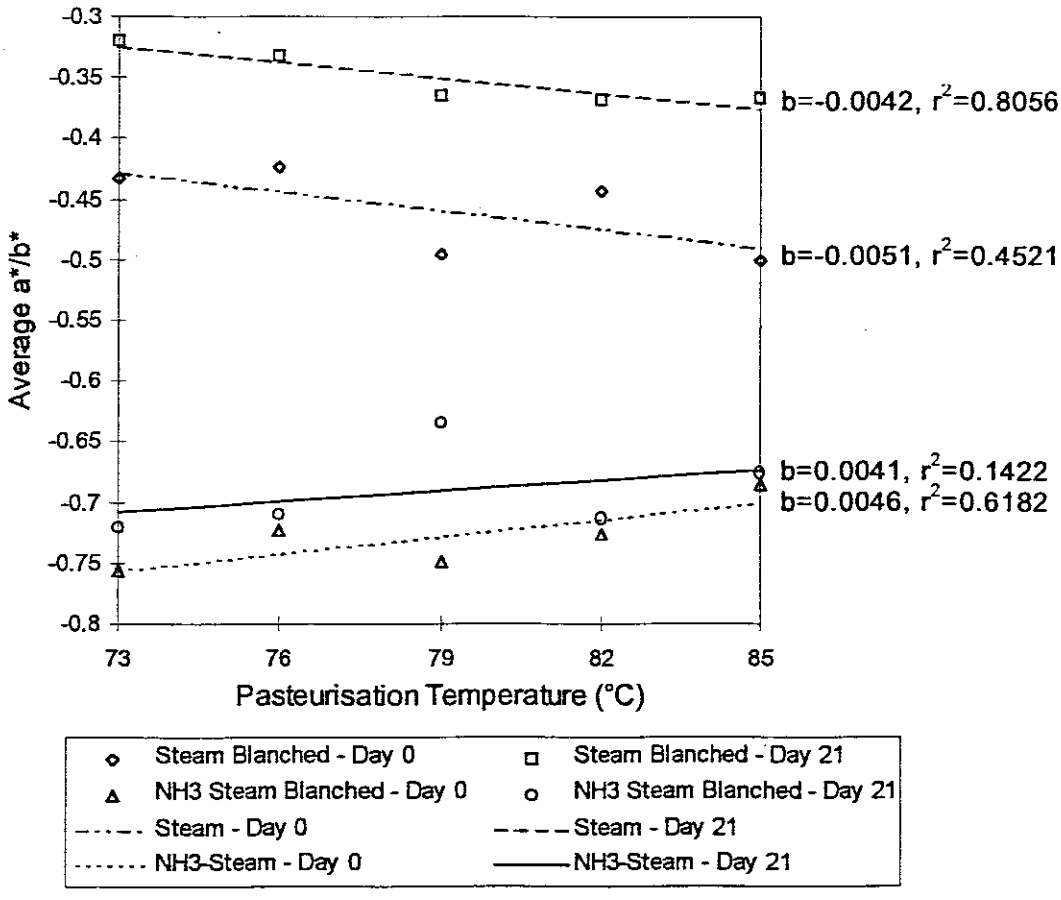

Figure 3.3 **Regression functions best fitting mean[a] a*/b* ratio values of sous-vide broccoli subjected to five heat treatments[b] and two blanching methods[c] at two storage periods[d].**

[a] Triplicate
[b] Pasteurisation temperatures: 73°, 76°, 79°, 82° and 85°C.
[c] Steam blanching and NH_3-steam blanching.
[d] Directly after sous-vide processing (Day 0) and after being stored for 21 days at 2 - 4°C (Day 21).

However, the a*/b* of NH_3-steam-blanched sous-vide broccoli did not change significantly after 21 days ($P>0.05$) storage at 2° - 4°C (Fig.3.3/Annexure 5.4).

Pasteurisation temperatures had no significant influence ($P>0.05$) on greenness of broccoli, except for steam blanched broccoli on Day 21 (Fig. 3.3/Annexure 5.5 - 5.8).

3.4.2 Hue angle (H°)

Mean hue angle (H°) values, of sous-vide broccoli directly after pasteurisation and again after 21 days cold storage at 2° - 4°C are shown in Figure 3.4. H° calculations substantiated the above a*b* ratio observations when significant deterioration ($P<0.05$) in greenness was observed with steam blanched broccoli being more yellow than NH_3-steam-blanched broccoli on both Day 0 and Day 21 (Fig. 3.4/Annexure 6.1 & 6.2). Steam blanched broccoli were significantly ($P<0.05$) more yellow on Day 21 than Day 0 (Fig.3.4/Annexure 6.3). NH_3-steam-blanched broccoli did not show a significant ($P>0.05$) difference in H° between samples from either day (Fig.3.4/Annexure 6.4).

Regression analysis indicated a significant influence ($P<0.05$) of pasteurisation temperatures on H° for steam blanched broccoli stored for 21 days at 2° - 4°C, with lower temperatures resulting in lower H°, and therefore more yellow broccoli (Fig. 3.4/Annexure 6.6).

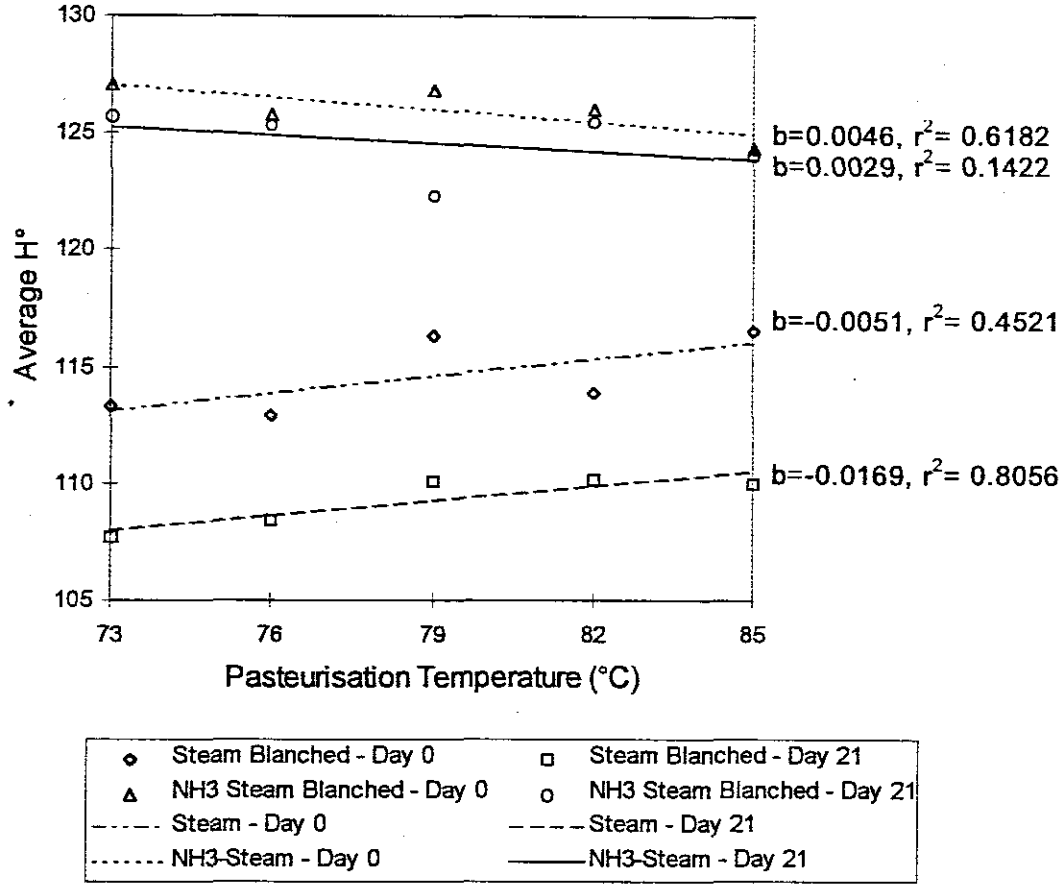

Figure 3.4 **Regression functions best fitting mean[a] hue angle values of sous-vide broccoli subjected to five heat treatments[b] and two blanching methods[c] at two storage periods[d].**

[a] Triplicate
[b] Pasteurisation temperatures: 73°, 76°, 79°, 82° and 85°C.
[c] Steam blanching and NH_3-steam blanching.
[d] Directly after sous-vide processing (Day 0) and after being stored for 21 days at 2 - 4°C (Day 21).

3.4.3 Chroma (C*)

The chroma (C*) values for sous-vide broccoli directly after pasteurisation and again after 21 days cold storage at 2° - 4°C are shown in Figure 3.5. No significant changes ($P>0.05$) in chroma for either of the blanching procedures on both Day 0 and Day 21 were observed (Fig. 3.5/Annexure 7.1 - 7.4).

No significant ($P>0.05$) effects of individual pasteurisation temperatures on chroma on Day 0 or Day 21 for NH_3-steam-blanched broccoli were detected (Fig. 3.5/Annexure 7.7 - 7.8). Steam blanched broccoli, however, showed a significant ($P<0.05$) increase in C* with an increase in pasteurisation temperatures on Day 21 (Fig. 3.5/Annexure 7.6). Steam blanched broccoli from Day 0 showed no significant ($P>0.05$) changes (Fig. 3.5/Annexure 7.5)

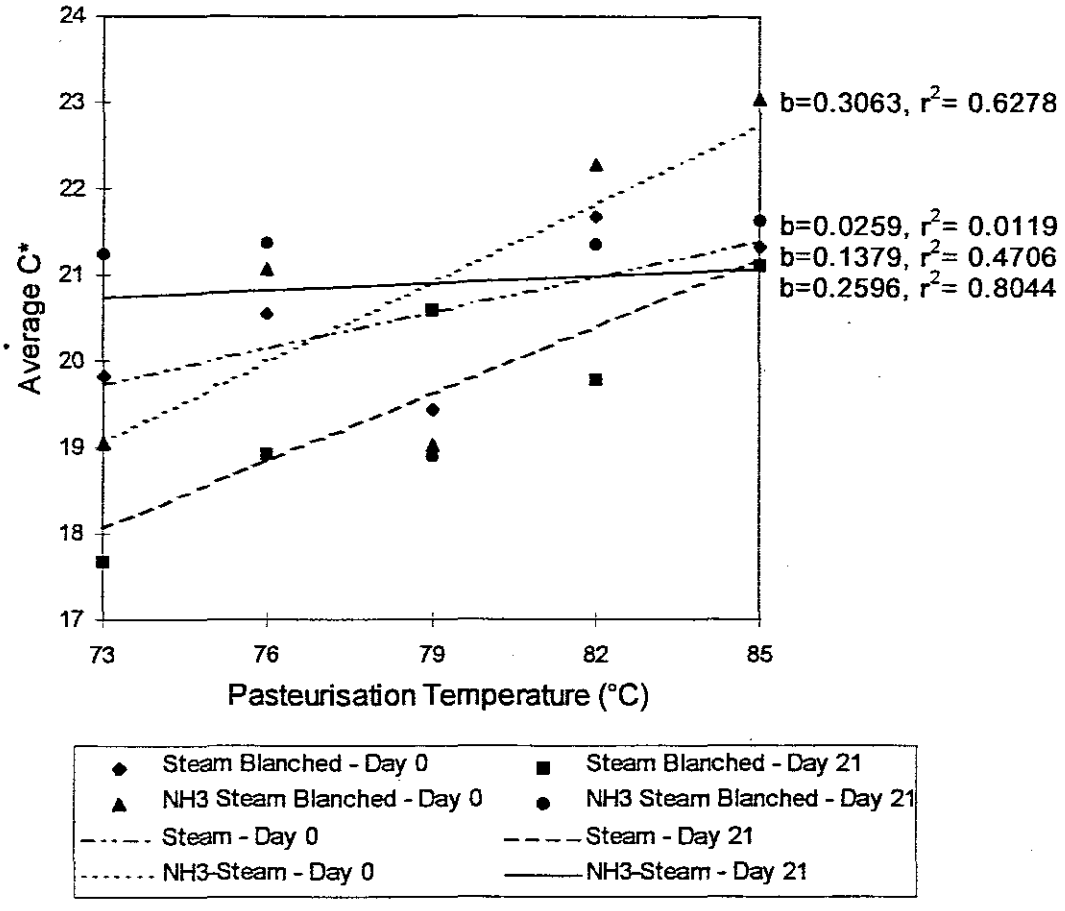

Figure 3.5 **Regression functions best fitting mean[a] chroma values of sous-vide broccoli subjected to five heat treatments[b] and two blanching methods[c] at two storage periods[d].**

[a] Triplicate
[b] Pasteurisation temperatures: 73°, 76°, 79°, 82° and 85°C.
[c] Steam blanching and NH_3-steam blanching.
[d] Directly after sous-vide processing (Day 0) and after being stored for 21 days at 2° - 4°C (Day 21).

3.5 Sensory analyses

The responses of the consumer panel of 30 panellists is tabulated in Table 3.2. The consumer panel indicated no significant taste preference ($P>0.05$) for broccoli from either of the two blanching procedures. NH_3-steam-blanched broccoli was preferred significantly ($P<0.05$) more than steam blanched broccoli in terms of colour but was judged to be significantly inferior in texture ($P<0.05$).

Table 3.2 Paired preference responses by a consumer panel of 30 members.

Blanching Procedure	Responses[1]		
	Taste perception	Colour acceptability	Texture
Steam	15a	6a	23a
NH_3-Steam	15a	24b	7b

[1] Values in a column having the same letter does not differ significantly ($P>0.05$)

CHAPTER 4

DISCUSSION

4.1 Heat penetration measurement

The first trials indicated that particular attention has to be paid to the packing gland integrity, as some of the bags lost their vacuum due to leaks at the packing gland, and no analyses could be carried out. This problem was aggravated during the insertion procedure of the thermocouple into its correct position. Precautionary steps were successfully introduced to overcome the problem by using a special heavy duty tape, Tesa Multikrafttape, able to withstand the processing conditions and mechanical strains. A section of tape was adhered to the outside of the bag over the area where the hole for the packing gland was to be made. This ensured a very strong bonding to the bag material, which permitted the successful insertion of the thermocouple without losing vacuum. The tape solved the problem and no further leaks were observed.

4.2 Pasteurisation

Pasteurisation temperatures of 73° up to 85°C in 3°C intervals were chosen to test the possible effects of temperature on the various quality aspects, while at the

same time investigating the practicality of reducing processing time by using higher temperatures. As the reference temperature was 70°C, temperatures higher than 70°C were selected in order to reduce pasteurisation time.

The study was successful in showing the significance of the various treatments from an overall quality perspective. In terms of optimising a sous-vide broccoli process, the first objective was to use quality raw materials. Secondly, broccoli was exposed to the same conditions prior and during the processing for all trials. However, being a natural product, certain aspects are impossible to dictate and therefore may influence the final product quality. For that reason all samples prepared for any replicate of the various analyses were cut from the same broccoli head. This procedure ensured reproducibility and assisted in showing that it is possible to predict quality aspects based on the effects of heat on the broccoli.

4.3 Analyses

4.3.1 pH-analysis

The significantly greater ($P<0.05$) loss in ascorbic acid of the NH_3-steam-blanched sous-vide broccoli is a direct result of the alkaline steam conditions that prevailed during blanching. Odland & Eheart (1975) also observed this increase in pH.

Higher pH values during this study, was the result of a relative small amount of broccoli exposed to the NH_3-vapour during blanching. A fairly constant flow of NH_3-gas evolved for at least 10 minutes after the NH_4HCO_3 solution was brought to the boil (Odland & Eheart, 1975). The liberation of NH_3 increased the pH of the steam and neutralised volatile acids present in the steam while at the same time neutralising acids present in the broccoli.

A further reason for the higher pH was the probable result of the design of the steam cabinet. The venting period of 3 minutes, to prevent the possible effects of a sudden rush of NH_3 - vapour allowed at the beginning of each NH_3-steam-blanching session, was possibly not enough. A solution could be constant controlled venting during the blanching process, to prevent build-up of NH_3 in the atmosphere inside. Alternatively, the amount of NH_4HCO_3, 2g.dm^{-3}, should be reduced. This was tried at first, but did not give repeatable results in terms of colour and was not considered further. Consistency in blanching treatments was therefore established by standardising procedures according to Odland & Eheart (1975).

4.3.2 Ascorbic acid analysis

Using the supernatant after centrifugation, as discussed in section 2.6.2, proved be quicker than the filtration methods. During the latter, the macerated broccoli tissue caused the

filter to block easily which resulted in unacceptable filtration times.

The lower ascorbic acid losses ($P<0.05$) with the increase in processing temperature are in line with findings by Dietz & Erdman (1989), who reported a significant decrease in retention of ascorbic acid in tomato juice with reduced sterilisation temperatures. The heat processing time increases as temperatures are reduced to achieve the same lethality. For sous-vide broccoli, in this study, a P_{70}^{10} of 100 was used throughout, with the result that the time of exposure to heat was less at higher temperatures. This is an important observation to ensure preservation of nutrients but at the same time effecting savings in processing time.

Petersen (1993) concluded that in processes eliminating leaching and oxidation ascorbic acid losses is decreased to such an extent that it becomes unsuitable as an indicator of vitamin retention. However, this study showed that steam blanched sous-vide broccoli retained significantly ($P<0.05$) more ascorbic acid than NH_3-steam-blanched broccoli after sous-vide processing. These findings are supported by that of Odland & Eheart (1975). It was therefore concluded that ascorbic acid content adequately indicates significant differences in effects of heat processing, and is useful as an indicator of nutrient retention.

4.3.3 Colour measurement.

Direct tristimulus values are less informative than the hue angle (H°) (Little, 1975). Both the a* and b* aspects are taken into account in calculating the H°. This explains the usefulness of H° as a single value when compared to individual tristimulus colour measurements. A change in H° indicates the direction in which the colour change took place. A change towards b*+ indicates a change towards yellow and a*- towards green.

The change in greenness through calculating the a*/b* ratio, also takes both a* and b* aspects of tristimulus analysis into account. The value thus obtained, proved to be sensitive enough in showing deterioration of colour towards yellow. The results of both analyses correlated in showing an increase in a*/b* values and decrease in H° of steam blanched sous-vide broccoli during storage for 21 days at 2° - 4°C, both indicating a move away from green to yellow. This change gave the broccoli a significantly unacceptable ($P<0.05$) yellow-green khaki-like appearance, as was shown by sensory analysis . This change in colour towards yellow is attributed to the conversion of chlorophyll to pheophytin as a result of the effects of acids present in the plant tissue as well as in the blanching steam.

Changes in colour when comparing the effects of the various pasteurisation temperatures on colour with each other, indicated little change ($P>0.05$) in a*/b* values or H°. Regression analysis showed for both a*/b* ratio and H°

analysis a significant ($P<0.05$) reduction in deterioration rate towards yellow at higher pasteurisation temperatures compared to lower temperatures, but only for steam blanched broccoli after 21 days storage at 2° - 4°C. These observations pointed out the suitability of either a*/b* ratio or H° in indicating degradation of green due to the effects of heat.

No significant effect ($P>0.05$) by either of the blanching procedures on the C* indicated a probable insensitivity of C* as an indicator of colour degradation due to heat processing in this temperature range.

4.3.4 Sensory analyses

The study by Odland & Eheart (1975) suggested frozen storage for broccoli after processing. Sensory analysis for that study did not include texture, after the frozen storage period. For this study, however, freezing was not considered, so to test the effects of blanching on texture and taste, sensory analysis was carried out only on Day 0. The consumer panel reiterated the fact that the consumer is constantly looking for better processed products that are as near to the fresh product as possible. Although the colour of the steam blanched product was rejected, the panel significantly ($P<0.05$) preferred the greenness of the NH_3-steam-blanched version. Comments made by the panel indicated that the bright green colour to be more appetising and fresh in appearance. Although not significant, some comments were made to the contrary stating the broccoli to

appear artificial. The statements by McLaren, (1980) and MacDougal (1984) relating to the importance of appearance as a quality attribute for food evaluation, especially by consumers, are corroborated by these results.

Texture suffered as a result of the higher pH and hence the steam blanched broccoli was preferred ($P<0.05$). The effects of the higher pH were predicted by Potter & Hotchkiss (1995). Odland & Eheart (1975) reported no significant difference in texture between steam blanched and NH_3-steam-blanched broccoli, but did mention a softer and more watery texture for NH_3-steam-blanched broccoli. As mentioned earlier, the possible reason for this difference in result could be the NH_3-steam-blanch procedure, including the equipment, that was employed. The inability of the panel to indicate significant ($P<0.05$) preference in taste showed that none of the blanching processes affects taste perception of sous-vide broccoli.

CHAPTER 5

CONCLUSIONS

This study showed the preference of consumers for products with acceptable colour and texture. The NH_3-steam-blanching procedure gave excellent colour by dramatically enhancing the greenness of sous-vide broccoli, but at the expense of both texture and ascorbic acid retention. The resulting higher pH is unacceptable from both a microbiological and a chemical perspective. This lead to the conclusion that the effects of NH_3-steam on texture and ascorbic acid retention need to be investigated and improved together with the actual methods employed and equipment used. NH_3-steam-blanching should only be considered if the effects of the procedure on texture are not important. Therefore, if nutrient retention is of paramount importance, steam blanching should be used. Water blanching can be considered for improved colour only if loss of water soluble nutrients are not important.

Although flavour is of great importance, the study also showed conclusively no preference for product from either blanching procedure. It can thus be concluded that the colour of sous-vide broccoli is not significantly affected by any of the pasteurisation processes employed.

This study has shown that processing time can successfully be reduced using higher temperatures for sous-vide

processing of broccoli and at the same time decrease ascorbic acid losses due to heat degradation. The range of temperatures used gave perspective to the times necessary for reaching the same P_v. At higher temperatures, ie. >82°C, the time needed was less than 7 - 8 minutes, depending on the thickness of the broccoli stem containing the thermocouple. Temperatures below 76°C will give times of >25 minutes, again depending on floret dimensions. The fact that 'pasteurisation temperatures did not influence quality, leads to the conclusion that it is possible to optimise a sous-vide process providing the equipment is adequate.

The best quality sous-vide broccoli will therefore be obtained under the following processing conditions:

- Use best possible quality broccoli.
- Apply HACCP procedures throughout processing to ensure control over processing conditions.
- Ensure uniform floret dimensions to be able to standardise on constant minimum heating process.
- Use steam blanching for maximum nutrient retention.
- Select a pasteurisation temperature between 76°C and 82°C to give a useful processing time.
- Ensure proper refrigerated storage after processing and monitor temperatures.
- Prevent cold spots in pasteurisation vessel by ensuring adequate mixing of heating medium, e.g. water.

CHAPTER 6

FUTURE RESEARCH

Although pH, ascorbic acid retention, and to a certain extent texture, were adversely affected by NH_3-steam blanching, there is room to minimise the effects by refining the procedures. The cabinet blanching equipment used in this study should be evaluated further to refine the NH_3-steam blanching method. Controlled venting, to prevent a build-up of NH_3 in the steam atmosphere, must be investigated. Alternatively, the amount of NH_4HCO_3 used in the water should be investigated and adjusted.

Future research should include further in-depth investigations into methods of enhancing the colour of broccoli, during prior blanching and without affecting product pH and texture, if it is to be used in sous-vide processing. Work should include investigation into the method used for colour enhancement, as well as the influence of the equipment used for this purpose.

Although 21 days is accepted to be the norm, the optimum storage time for this pack must be established in terms of sensory quality as well as microbiological stability. Ultra short pasteurisation procedures must be investigated in order to reduce the effects of heat processes on texture. A continuous system should be used, when reducing the pasteurisation

times, but at the same time maintain control over the pasteurisation integrity of the packs.

Mathematical optimisation models as described by Ghazala & Aucoin (1996) should be employed once the effects of colour enhancement have been reduced.

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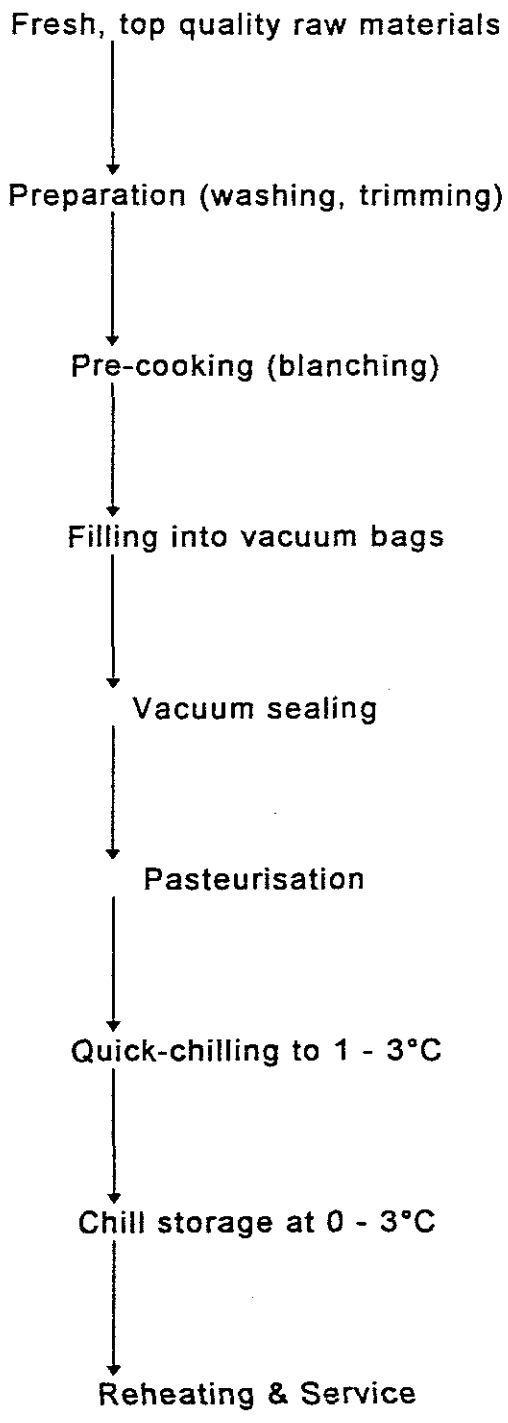
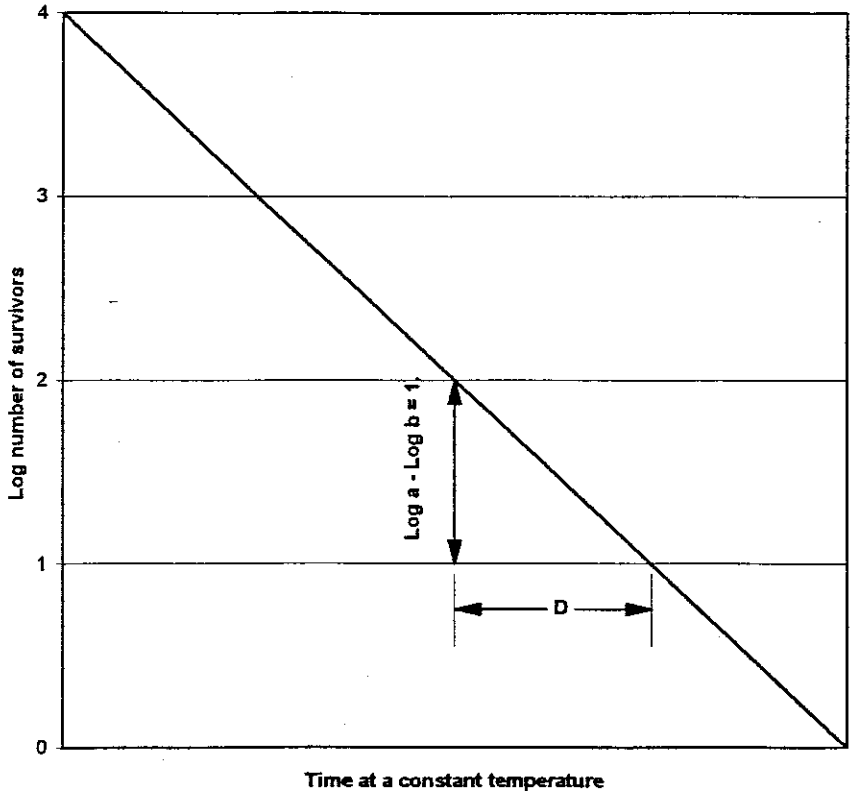
INDEX TO ANNEXURES:

Annexure 1: Basic Sous-Vide Cooking Process.



Annexure 2: Bacterial Destruction Rate Curve showing logarithmic order of death.



Adapted from Stumbo (1973) and Potter & Hotchkiss (1995)

Annexure 3.1: Analysis of variance: pH Measurements

Steam blanched vs NH_3-Steam blanched sous-vide broccoli - Day 0

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Steam Blanched - Day 0	5	33.12667	6.625333	0.002581
NH3 Steam Blanched - Day 0	5	37.94333	7.588667	0.008203
73°C	2	14.08	7.04	0.405
76°C	2	14.19333	7.096667	0.3872
79°C	2	14.23667	7.118333	0.408006
82°C	2	14.28	7.14	0.4608
85°C	2	14.28	7.14	0.688356

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	2.320028	1	2.320028	316.3674	5.87E-05	7.70865
Columns	0.013804	4	0.003451	0.470606	0.758325	6.388234
Error	0.029333	4	0.007333			
Total	2.363166	9				

Annexure 3.2 Analysis of variance: pH Measurements

Steam blanched vs NH_3-Steam blanched sous-vide broccoli - Day 21

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Steam Blanched - Day 21	5	33.04	6.608	0.00372
NH3 Steam Blanched - Day 21	5	37.3	7.46	0.001161
73°C	2	14.00667	7.003333	0.358422
76°C	2	14.1	7.05	0.3362
79°C	2	14.10667	7.053333	0.278756
82°C	2	14.10333	7.051667	0.372672
85°C	2	14.02333	7.011667	0.483472

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	1.81476	1	1.81476	491.7308	2.45E-05	7.70865
Columns	0.004762	4	0.001191	0.322595	0.850544	6.388234
Error	0.014762	4	0.003691			
Total	1.834284	9				

Annexure 3.3: Analysis of variance: pH Measurements

Steam blanched sous-vide broccoli - Day 0 vs Day 21

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Steam Blanched - Day 0	5	33.12667	6.625333	0.002581
Steam Blanched - Day 21	5	33.04	6.608	0.00372
73°C	2	13.17	6.585	5E-05
76°C	2	13.29667	6.648333	0.000139
79°C	2	13.34667	6.673333	8.89E-05
82°C	2	13.28	6.64	0.0008
85°C	2	13.07333	6.536667	0.000556

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.000751	1	0.000751	3.405542	0.138725	7.70865
Columns	0.024322	4	0.006081	27.56927	0.00359	6.388234
Error	0.000882	4	0.000221			
Total	0.025956	9				

Annexure 3.4: Analysis of variance: pH Measurements

NH_3-Steam blanched sous-vide broccoli - Day 0 vs Day 21

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
NH3 Steam Blanched - Day 0	5	37.94333	7.588667	0.008203
NH3 Steam Blanched - Day 21	5	37.3	7.46	0.001161
73°C	2	14.91667	7.458333	0.002006
76°C	2	14.99667	7.498333	0.002939
79°C	2	14.99667	7.498333	0.010272
82°C	2	15.10333	7.551667	0.009339
85°C	2	15.23	7.615	0.024939

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.041388	1	0.041388	20.4216	0.010666	7.70865
Columns	0.029351	4	0.007338	3.620614	0.120241	6.388234
Error	0.008107	4	0.002027			
Total	0.078846	9				

Annexure 3.5: **Linear regression analysis - pH values**
Steam Blanched Broccoli - Day 0

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.21785358
R Square	0.04746018
Adjusted R Square	-0.2700531
Standard Error	0.05725511
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.00049	0.0005	0.14947	0.724830185
Residual	3	0.009834444	0.0033		
Total	4	0.010324444			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	6.80966667	0.477469362	14.262	0.00075	5.290144634	8.3291887
Pasteurisation temperature (°C)	-0.0023333	0.006035219	-0.387	0.72483	-0.02154011	0.0168734

Annexure 3.6: **Linear regression analysis - pH values**
Steam Blanched Broccoli - Day 21

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.36293309
R Square	0.13172043
Adjusted R Square	-0.1577061
Standard Error	0.0656252
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.00196	0.002	0.45511	0.548254441
Residual	3	0.01292	0.0043		
Total	4	0.01488			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	6.97666667	0.547270263	12.748	0.00104	5.235006806	8.7183265
Pasteurisation temperature (°C)	-0.0046667	0.006917503	-0.675	0.54825	-0.02668127	0.0173479

Annexure 3.7: **Linear regression analysis - pH values**
NH_3-Steam Blanched Broccoli - Day 0

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.9717847
R Square	0.9443654
Adjusted R Square	0.9258206
Standard Error	0.0246682
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.030987778	0.031	50.9233	0.0056652
Residual	3	0.001825556	0.0006		
Total	4	0.032813333			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	6.1227778	0.205716023	29.763	8.3E-05	5.46809696	6.7774586
Pasteurisation temperature (°C)	0.0185556	0.002600253	7.1361	0.00567	0.01028038	0.0268307

Annexure 3.8: **Linear regression analysis - pH values**
NH_3-Steam Blanched Broccoli - Day 21

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.8197619
R Square	0.6720096
Adjusted R Square	0.5626794
Standard Error	0.0225339
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.003121111	0.0031	6.14661	0.0893304
Residual	3	0.001523333	0.0005		
Total	4	0.004644444			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	6.9947778	0.187917864	37.223	4.3E-05	6.39673871	7.5928168
Pasteurisation temperature (°C)	0.0058889	0.002375284	2.4792	0.08933	-0.00167033	0.0134481

Annexure 4.1: Analysis of variance: Ascorbic Acid Losses (%)

Steam blanched vs NH_3-blanched sous-vide broccoli - Day 0

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Steam Blanched - Day 0	5	230.72	46.144	44.92553
NH3 Steam Blanched - Day 0	5	259.27	51.854	46.51283
73 °C	2	113.23	56.615	17.58245
76°C	2	106.71	53.355	19.40645
79 °C	2	100.65	50.325	12.45005
82 °C	2	90.27	45.135	16.30205
85 °C	2	79.13	39.565	16.18805

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	81.51025	1	81.51025	778.5124	9.82E-06	7.70865
Columns	365.3346	4	91.33366	872.3368	3.93E-06	6.388234
Error	0.4188	4	0.1047			
Total	447.2637	9				

Annexure 4.2: **Analysis of variance: Ascorbic Acid Losses (%)**

Steam blanched vs NH_3-blanched sous-vide broccoli - Day 21

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Steam Blanched - Day 21	5	244.97	48.994	13.90163
NH_3 Steam Blanched - Day 21	5	265.79	53.158	20.10117
73 °C	2	112.91	56.455	19.78205
76°C	2	105.32	52.66	6.6978
79 °C	2	101.23	50.615	21.97845
82 °C	2	98.78	49.39	0.72
85 °C	2	92.52	46.26	14.7968

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	43.34724	1	43.34724	8.405572	0.044152	7.70865
Columns	115.3833	4	28.84583	5.593568	0.062028	6.388234
Error	20.62786	4	5.156965			
Total	179.3584	9				

Annexure 4.3: Analysis of variance: Ascorbic Acid Losses (%)

Steam blanched sous-vide broccoli - Day 0 vs Day 21

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Steam Blanched - Day 0	5	230.72	46.144	44.92553
Steam Blanched - Day 21	5	244.97	48.994	13.90163
73 °C	2	106.96	53.48	0.0578
76°C	2	101.07	50.535	0.17405
79 °C	2	95.13	47.565	0.14045
82 °C	2	92.27	46.135	29.72205
85 °C	2	80.26	40.13	23.2562

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	20.30625	1	20.30625	2.458064	0.191995	7.70865
Columns	202.2643	4	50.56608	6.121005	0.053623	6.388234
Error	33.0443	4	8.261075			
Total	255.6149	9				

Annexure 4.4: **Analysis of variance: Ascorbic Acid Losses (%)**

NH_3-Steam blanched sous-vide broccoli - Day 0 vs Day 21

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
NH_3 Steam Blanched - Day 0	5	259.27	51.854	46.51283
NH_3 Steam Blanched - Day 21	5	265.79	53.158	20.10117
73 °C	2	119.18	59.59	0.0002
76°C	2	110.96	55.48	1.9602
79 °C	2	106.75	53.375	0.61605
82 °C	2	96.78	48.39	0.32
85 °C	2	91.39	45.695	21.58245

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	4.25104	1	4.25104	0.840631	0.411094	7.70865
Columns	246.2281	4	61.55703	12.17272	0.016414	6.388234
Error	20.22786	4	5.056965			
Total	270.707	9				

Annexure 4.5: Linear regression analysis - % Ascorbic Acid Loss Steam Blanched Broccoli - Day 0

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.9855768
R Square	0.9713616
Adjusted R Square	0.9618155
Standard Error	1.3014002
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	172.335802	172.3	101.75	0.00207484
Residual	3	5.080927784	1.694		
Total	4	177.4167298			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	155.43079	10.85280762	14.32	0.0007	120.8922762	189.9693
Pasteurisation Temperature (°C)	-1.3837782	0.137179631	-10.1	0.0021	-1.82034547	-0.947211

Annexure 4.6: Linear regression analysis - % Ascorbic Acid Loss Steam Blanched Broccoli - Day 21

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.9598529
R Square	0.9213175
Adjusted R Square	0.8950901
Standard Error	1.1938617
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	50.06805617	50.07	35.128	0.009598043
Residual	3	4.275916957	1.425		
Total	4	54.34397313			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	107.55195	9.956007722	10.8	0.0017	75.86746264	139.23644
Pasteurisation Temperature (°C)	-0.7458631	0.125844068	-5.93	0.0096	-1.14635545	-0.345371

Annexure 4.7: Linear regression analysis - % Ascorbic Acid Loss
NH_3-Steam Blanched Broccoli - Day 0

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.9927849
R Square	0.9856219
Adjusted R Square	0.9808293
Standard Error	0.9439322
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	183.2369346	183.2	205.65	0.000734891
Residual	3	2.673024202	0.891		
Total	4	185.9099588			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	164.57955	7.871763528	20.91	0.0002	139.528058	189.631
Pasteurisation Temperature (°C)	-1.4268728	0.099499194	-14.3	0.0007	-1.74352392	-1.11022

Annexure 4.8: Linear regression analysis - % Ascorbic Acid Loss
NH_3-Steam Blanched Broccoli - Day 21

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.8927677
R Square	0.7970341
Adjusted R Square	0.7293788
Standard Error	1.938549
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	44.27195509	44.27	11.781	0.041467769
Residual	3	11.27391709	3.758		
Total	4	55.54587218			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	113.52101	16.16620241	7.022	0.0059	62.07288669	164.9691
Pasteurisation Temperature (°C)	-0.7013634	0.20434101	-3.43	0.0415	-1.3516683	-0.05106

Annexure 5.1: Analysis of variance: a*/b* Ratios

Steam blanched vs NH_3-blanched sous-vide broccoli - Day 0

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Steam Blanched - Day 0	5	-2.29594	-0.45919	0.0013
NH_3 Steam Blanched - Day 0	5	-3.64014	-0.72803	0.000779
73°C	2	-1.18913	-0.59456	0.052175
76°C	2	-1.14707	-0.57354	0.044812
79°C	2	-1.24499	-0.62249	0.032094
82°C	2	-1.16989	-0.58495	0.040205
85°C	2	-1.185	-0.5925	0.017089

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.180687	1	0.180687	127.0688	0.000353	7.70865
Columns	0.002629	4	0.000657	0.462188	0.76342	6.388234
Error	0.005688	4	0.001422			
Total	0.189004	9				

Annexure 5.2: Analysis of variance: a*/b* Ratios

Steam blanched vs NH_3-blanched sous-vide broccoli - Day 21

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Steam Blanched - Day 21	5	-1.75023	-0.35005	0.000497
NH_3 Steam Blanched - Day 21	5	-3.45128	-0.69026	0.001294
73°C	2	-1.04028	-0.52014	0.080044
76°C	2	-1.0412	-0.5206	0.071054
79°C	2	-0.99868	-0.49934	0.036121
82°C	2	-1.08023	-0.54011	0.059684
85°C	2	-1.04112	-0.52056	0.047953

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.289357	1	0.289357	210.4163	0.000131	7.70865
Columns	0.001664	4	0.000416	0.302483	0.86325	6.388234
Error	0.005501	4	0.001375			
Total	0.296521	9				

Annexure 5.3: Analysis of variance: a*/b* Ratios

Steam blanched sous-vide broccoli - Day 0 vs Day 21

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Steam Blanched - Day 0	5	-2.29594	-0.45919	0.0013
Steam Blanched - Day 21	5	-1.75023	-0.35005	0.000497
73°C	2	-0.75313	-0.37657	0.006381
76°C	2	-0.75597	-0.37798	0.004208
79°C	2	-0.86077	-0.43038	0.008563
82°C	2	-0.81053	-0.40526	0.002873
85°C	2	-0.86578	-0.43289	0.009025

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.02978	1	0.02978	93.91936	0.000634	7.70865
Columns	0.00592	4	0.00148	4.667314	0.082417	6.388234
Error	0.001268	4	0.000317			
Total	0.036968	9				

Annexure 5.4 Analysis of variance: a*/b* Ratios

NH_3-Steam blanched sous-vide broccoli - Day 0 vs Day 21

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
NH_3 Steam Blanched - Day 0	5	-3.64014	-0.72803	0.000779
NH_3 Steam Blanched - Day 21	5	-3.45128	-0.69026	0.001294
73°C	2	-1.47627	-0.73814	0.000644
76°C	2	-1.43231	-0.71615	9.99E-05
79°C	2	-1.3829	-0.69145	0.006663
82°C	2	-1.43959	-0.7198	9.61E-05
85°C	2	-1.36034	-0.68017	4.55E-05

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.003567	1	0.003567	3.583374	0.131298	7.70865
Columns	0.004311	4	0.001078	1.082828	0.47019	6.388234
Error	0.003982	4	0.000995			
Total	0.01186	9				

Annexure 5.5: **Linear regression analysis - a*/b* Ratios**
Steam Blanched Broccoli - Day 0

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.67236451
R Square	0.45207404
Adjusted R Square	0.26943205
Standard Error	0.03082131
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.002351316	0.002	2.47519	0.213713265
Residual	3	0.002849859	9E-04		
Total	4	0.005201175			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.0553927	0.257029086	-0.216	0.84319	-0.87337476	0.762589
Pasteurisation Temperature (°C)	-0.0051113	0.003248851	-1.573	0.21371	-0.01545064	0.005228

Annexure 5.6: **Linear regression analysis - a*/b* Ratios**
Steam Blanched Broccoli - Day 21

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.89752482
R Square	0.80555081
Adjusted R Square	0.74073441
Standard Error	0.0113483
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.001600552	0.002	12.4282	0.038767792
Residual	3	0.000386352	1E-04		
Total	4	0.001986903			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.0168946	0.094637233	-0.179	0.86969	-0.3180728	0.284284
Pasteurisation Temperature (°C)	-0.0042171	0.001196216	-3.525	0.03877	-0.00802399	-0.00041

Annexure 5.7: **Linear regression analysis - a*/b* Ratios**
NH_3-Steam Blanched Broccoli - Day 0

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.78624
R Square	0.6181733
Adjusted R Square	0.4908978
Standard Error	0.0199131
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.001925937	0.002	4.857	0.1147581
Residual	3	0.001189592	4E-04		
Total	4	0.003115529			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-1.0934769	0.166061747	-6.585	0.0071	-1.62196	-0.564994
Pasteurisation Temperature (°C)	0.0046259	0.002099023	2.204	0.1148	-0.002054	0.011306

Annexure 5.8: **Linear regression analysis - a*/b* Ratios**
NH_3-Steam Blanched Broccoli - Day 21

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.3770982
R Square	0.142203
Adjusted R Square	-0.1437293
Standard Error	0.0384766
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.000736272	7E-04	0.4973	0.5314991
Residual	3	0.004441338	0.001		
Total	4	0.00517761			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.9162117	0.320868809	-2.855	0.0648	-1.93736	0.104937
Pasteurisation Temperature (°C)	0.0028602	0.004055786	0.705	0.5315	-0.010047	0.015768

Annexure 6.1: Analysis of variance: Hue angle (H°)

Steam blanched vs NH_3-blanched sous-vide broccoli - Day 0

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Steam Blanched - Day 0	5	573.078	114.6156	2.92955
NH_3 Steam Blanched - Day 0	5	630.1469	126.0294	1.126455
73°C	2	240.432	120.216	94.54872
76°C	2	238.7553	119.3777	82.95041
79°C	2	243.1755	121.5878	55.15215
82°C	2	239.9036	119.9518	73.28479
85°C	2	240.9584	120.4792	30.64111

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	325.687	1	325.687	119.626	0.000397	7.70865
Columns	5.333843	4	1.333461	0.489785	0.746815	6.388234
Error	10.89018	4	2.722544			
Total	341.911	9				

Annexure 6.2: Analysis of variance: Hue angle (H°)

Steam blanched vs NH_3-blanched sous-vide broccoli - Day 21

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Steam Blanched - Day 21	5	546.3084	109.2617	1.283661
NH_3 Steam Blanched - Day 21	5	622.9625	124.5925	1.993208
73°C	2	233.4726	116.7363	162.3081
76°C	2	233.7061	116.853	144.0312
79°C	2	232.3982	116.1991	75.91907
82°C	2	235.6514	117.8257	117.2762
85°C	2	234.0426	117.0213	98.38164

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	587.5853	1	587.5853	227.5043	0.000113	7.70865
Columns	2.7765	4	0.694125	0.268755	0.884399	6.388234
Error	10.33098	4	2.582744			
Total	600.6928	9				

Annexure 6.3: Analysis of variance: Hue angle (H°)

Steam blanched sous-vide broccoli - Day 0 vs Day 21

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Steam Blanched - Day 0	5	573.078	114.6156	2.92955
Steam Blanched - Day 21	5	546.3084	109.2617	1.283661
73°C	2	221.0681	110.534	15.75077
76°C	2	221.3044	110.6522	10.44575
79°C	2	226.3744	113.1872	19.83553
82°C	2	224.0667	112.0333	6.957729
85°C	2	226.5728	113.2864	21.49963

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	71.66098	1	71.66098	101.3435	0.000548	7.70865
Columns	14.0244	4	3.506101	4.958355	0.075048	6.388234
Error	2.828438	4	0.70711			
Total	88.51383	9				

Annexure 6.4: Analysis of variance: Hue angle (H°)

NH_3-Steam blanched sous-vide broccoli - Day 0 vs Day 21

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
NH_3 Steam Blanched - Day 0	5	630.1469	126.0294	1.126455
NH_3 Steam Blanched - Day 21	5	622.9625	124.5925	1.993208
73°C	2	252.8365	126.4182	0.906914
76°C	2	251.157	125.5785	0.114515
79°C	2	249.1993	124.5996	10.02992
82°C	2	251.4884	125.7442	0.136153
85°C	2	248.4283	124.2141	0.064241

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	5.161634	1	5.161634	3.390175	0.139397	7.70865
Columns	6.388544	4	1.597136	1.049003	0.482067	6.388234
Error	6.09011	4	1.522527			
Total	17.64029	9				

Annexure 6.5: **Linear regression analysis - Hue Angle (H°)**
Steam Blanched Broccoli - Day 0

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.6723645
R Square	0.452074
Adjusted R Square	0.2694321
Standard Error	0.0308213
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.002351316	0.002	2.47519	0.213713265
Residual	3	0.002849859	9E-04		
Total	4	0.005201175			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.0553927	0.257029086	-0.216	0.84319	-0.873374755	0.762589
Pasteurisation Temperature (°C)	-0.0051113	0.003248851	-1.573	0.21371	-0.015450638	0.005228

Annexure 6.6: **Linear regression analysis - Hue Angle (H°)**
Steam Blanched Broccoli - Day 21

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.8975248
R Square	0.8055508
Adjusted R Square	0.7407344
Standard Error	0.0113483
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.001600552	0.002	12.4282	0.038767792
Residual	3	0.000386352	1E-04		
Total	4	0.001986903			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.0168946	0.094637233	-0.179	0.86969	-0.3180728	0.284284
Pasteurisation Temperature (°C)	-0.0042171	0.001196216	-3.525	0.03877	-0.008023993	-0.00041

Annexure 6.7: **Linear regression analysis - Hue Angle (H°)**
NH_3-Steam Blanched Broccoli - Day 0

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.78624
R Square	0.6181733
Adjusted R Square	0.4908978
Standard Error	0.0199131
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.001925937	0.002	4.857	0.114758126
Residual	3	0.001189592	4E-04		
Total	4	0.003115529			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-1.0934769	0.166061747	-6.585	0.0071	-1.62196002	-0.564994
Pasteurisation Temperature (°C)	0.0046259	0.002099023	2.204	0.1148	-0.0020541	0.011306

Annexure 6.8: **Linear regression analysis - Hue Angle (H°)**
NH_3-Steam Blanched Broccoli - Day 21

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.3770982
R Square	0.142203
Adjusted R Square	-0.1437293
Standard Error	0.0384766
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.000736272	7E-04	0.4973	0.531499144
Residual	3	0.004441338	0.001		
Total	4	0.00517761			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.9162117	0.320868809	-2.855	0.0648	-1.93736038	0.104937
Pasteurisation Temperature (°C)	0.0028602	0.004055786	0.705	0.5315	-0.01004712	0.015768

Annexure 7.1: Analysis of variance: Chroma (C*)

Steam blanched vs NH_3-blanched sous-vide broccoli - Day 0

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Steam Blanched - Day 0	5	102.7797	20.55594	0.908888
NH_3 Steam Blanched - Day 0	5	104.4782	20.89564	3.361662
73°C	2	38.87984	19.43992	0.297464
76°C	2	41.60681	20.80341	0.144802
79°C	2	38.45365	19.22682	0.079781
82°C	2	43.94464	21.97232	0.189445
85°C	2	44.37293	22.18647	1.471612

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.288492	1	0.288492	0.609078	0.478738	7.70865
Columns	15.18758	4	3.796896	8.016196	0.034175	6.388234
Error	1.894612	4	0.473653			
Total	17.37069	9				

Annexure 7.2: **Analysis of variance: Chroma (C*)**

Steam blanched vs NH_3-blanched sous-vide broccoli - Day 21

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Steam Blanched - Day 21	5	98.07224	19.61445	1.885621
NH_3 Steam Blanched - Day 21	5	104.5028	20.90057	1.27798
73°C	2	38.90527	19.45263	6.380861
76°C	2	40.28162	20.14081	3.053722
79°C	2	39.48661	19.74331	1.437369
82°C	2	41.14331	20.57165	1.215722
85°C	2	42.75827	21.37914	0.136822

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	4.135253	1	4.135253	2.044816	0.225951	7.70865
Columns	4.565163	4	1.141291	0.56435	0.703465	6.388234
Error	8.089243	4	2.022311			
Total	16.78966	9				

Annexure 7.3: Analysis of variance: Chroma (C*)

Steam blanched sous-vide broccoli - Day 0 vs Day 21

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Steam Blanched - Day 0	5	102.7797	20.55594	0.908888
Steam Blanched - Day 21	5	98.07224	19.61445	1.885621
73°C	2	37.49204	18.74602	2.33091
76°C	2	39.43948	19.71974	1.327121
79°C	2	40.01761	20.0088	0.678042
82°C	2	41.45655	20.72827	1.75322
85°C	2	42.44625	21.22313	0.02228

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	2.216001	1	2.216001	2.275405	0.205935	7.70865
Columns	7.282462	4	1.820616	1.86942	0.279708	6.388234
Error	3.895573	4	0.973893			
Total	13.39404	9				

Annexure 7.4: Analysis of variance: Chroma (C*)

NH_3-Steam blanched sous-vide broccoli - Day 0 vs Day 21

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
NH_3 Steam Blanched - Day 0	5	104.4782	20.89564	3.361662
NH_3 Steam Blanched - Day 21	5	104.5028	20.90057	1.27798
73°C	2	40.29307	20.14654	2.386121
76°C	2	42.44895	21.22448	0.046205
79°C	2	37.92265	18.96133	0.008652
82°C	2	43.6314	21.8157	0.431317
85°C	2	44.68495	22.34247	0.985

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	6.07E-05	1	6.07E-05	6.3E-05	0.994048	7.70865
Columns	14.70133	4	3.675333	3.811366	0.111637	6.388234
Error	3.857235	4	0.964309			
Total	18.55863	9				

Annexure 7.5: Linear regression analysis - Chroma (C*)
Steam Blanched Broccoli - Day 0

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.6860222
R Square	0.4706264
Adjusted R Square	0.2941686
Standard Error	0.8009503
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.710985967	1.711	2.6671	0.200950429
Residual	3	1.924564056	0.642		
Total	4	3.635550023			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	9.6633993	6.679389644	1.447	0.2438	-11.5934195	30.92022
Pasteurisation Temperature (°C)	0.1378802	0.084427573	1.633	0.201	-0.13080625	0.406567

Annexure 7.6: Linear regression analysis - Chroma (C*)
Steam Blanched Broccoli - Day 21

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.8968715
R Square	0.8043786
Adjusted R Square	0.7391714
Standard Error	0.7013016
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	6.067013327	6.067	12.336	0.039135129
Residual	3	1.475471719	0.492		
Total	4	7.542485046			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.8968576	5.848386131	-0.15	0.8879	-19.5090499	17.71533
Pasteurisation Temperature (°C)	0.2596368	0.073923677	3.512	0.0391	0.024378429	0.494895

Annexure 7.7: **Linear regression analysis - Chroma (C*)**
NH_3-Steam Blanched Broccoli - Day 0

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.7923096
R Square	0.6277545
Adjusted R Square	0.5036726
Standard Error	1.2916984
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	8.441192674	8.441	5.0592	0.110012853
Residual	3	5.005454421	1.668		
Total	4	13.4466471			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-3.2983661	10.7719009	-0.31	0.7795	-37.57939443	30.982662
Pasteurisation Temperature (°C)	0.3062532	0.136156969	2.249	0.11	-0.127059436	0.7395659

Annexure 7.8: **Linear regression analysis - Chroma (C*)**
NH_3-Steam Blanched Broccoli - Day 0

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.1088983
R Square	0.0118588
Adjusted R Square	-0.3175215
Standard Error	1.2976002
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.060621452	0.061	0.036	0.861620905
Residual	3	5.051298916	1.684		
Total	4	5.111920368			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	18.850259	10.82111789	1.742	0.1799	-15.58739952	53.287918
Pasteurisation Temperature (°C)	0.0259533	0.136779072	0.19	0.8616	-0.409339201	0.4612457