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PRELIMINARY OBSERVATIONS ON NUTRITIONAL AND MICROBIOLOGICAL CHANGES OF HOT AND COLD SMOKED TROUT *(ONCORHYNCHUS MYKISS)*

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ABSTRACT

The objective of this study is to compare nutritional, chemical and microbial changes during processing and subsequent storage at 6°C of hot and cold smoked rainbow trout (*Oncorhynchus mykiss)*. The fish was smoked using two different methods: cold smoking according to usual procedures in Iceland and hot smoking similar to the Iranian tradition. For chemical variation fat, protein, salt, ash and water content were measured during processing as well as weight changes. Quality changes during storage were monitored by measuring the TVB content and microbial load.

Significant weight and water losses were observed in both hot and cold smoking. As a result, protein and salt increased significantly especially in the hot smoking process. The quality evaluation (microbiological and chemical variation for cold smoked fish) indicated that the fish wasn't acceptable after two weeks of storage at 6°C. Cold smoked fillet of rainbow trout is more susceptible to microbial spoilage than whole hot smoked fish. Rainbow trout is suitable for hot smoking and has good nutritional value and keeping quality.

LIST OF ABBREVIATIONS

AOAC	:	Association of Official Analytical Chemists
APHA	:	American Public Health Association
DIFCO	:	Becton Dickinson and company of France, Microbiology system
FAO	:	Food and Agriculture Organization
IFT	:	Institute of Food Technologists
ISO	:	International Standard Organization
IJFM	:	International journal of Food Microbiology
LAB	:	Lactic acid bacteria
OST	:	Office of Science and Technology
PDO	:	Planning and Development Office Fisheries of Iran
RH	:	Relative Humidity
TPC	:	Total plate count
TVB	:	Total volatile basic amine
BGLB	:	Brilliant Green Lactose Bile Broth
EC	:	Escherichia coli
MPN	:	Most Probable Number
LST	:	Lauryl Sulphate Tryptose
CEJA	:	Council of the European Young Farmers
TRS	:	Tory Research Station
TPC	:	Total plate count
MRS	:	De Man, Rogosa, Sharpe. A solidified version of MRS broth for the culture of Lactic acid bacteria.

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1 INTRODUCTION

Iran has a coastline of more than 1800 km along the Persian Gulf and the Oman Sea and 900 km along the Caspian Sea with considerable potential for harvesting from living aquatic resources. At present, the average per capita fish consumption in Iran is low, at around 4.7 kg compared with the world average of 13.5 kg (OST 2003). However to ensure national food security and to compensate the regulatory limitations in fish catch, Iranian fisheries have tried to increase the aquaculture production of commercially valuable species. In 2003 the production of fish in Iran was 448,000 metric tons, 374,000 (83%) tons were captured and 74,000(17%) came from aquaculture (PDO 2004).

Aquaculture in Iran started in 1922. The importance of aquaculture has increased and it is anticipated that the sector will expand further. Traditional aquaculture fish species include silver carp, grass carp and common carp (Rana and Bartley 1998). One of the more recent species is rainbow trout (*Oncorhynchus mykiss*). Production of rainbow trout started in 1956. In 1999 the production was 7000 tons, and reached 9000 tons in 2000 (Mehrabi 2002). The production of rainbow trout during the years has been constant and it is mostly sold fresh. However, during peak supply trout must be processed to preserve its quality because it is a very perishable product. Different methods like chilling, freezing and smoking are commonly used for preservation.

In Iran there is a long tradition of smoking for preservation but also for the appealing taste of the smoked product (Razavi 1994). Very different methods for smoking are used in Iran compared to the Nordic countries. Smoking in Iran is mostly applied to fish species like *Rutilus frisii kutum* but sometimes rainbow trout is used.

Cold smoked fish typically has a shelf life of two to three weeks in chilled storage (<4°C) but hot smoked products have a longer shelf life. Cold smoked rainbow trout by the Scandinavian method is not well known in Iran. On the other hand, hot smoked rainbow trout is not well known in many countries outside of Iran, including Iceland.

The hot smoking process is rather harsh compared to cold smoking. The fish is salted, heated and dried. This process markedly affects the chemical and microbial status of the product. It can lower the nutritional value but at the same time prolong the shelf life. The shelf life of cold smoked fish is basically limited by microbial activity while the shelf life of the hot smoked fish can be affected by lipid oxidation (Bligh *et al.* 1988).

The successful introduction of rainbow trout to the Iranian market will be supported by having alternative and accepted methods for post-harvest processing. The acceptance will be based on documented knowledge on the quality and safety of these products. Vacuum packaging can prolong the shelf life of food, including fish, by lowering the amount of oxygen and thereby restraining all processes requiring oxygen like aerobic growth of bacteria and lipid oxidation. There are numerous reports on cold smoking of fish especially salmon and trout, while very little is known about hot smoking of rainbow trout (Lyhs 2002).

Therefore, the aim of this project is to compare hot and cold smoking of rainbow trout in terms of chemical changes during processing and microbial changes during subsequent storage. A further aim is to study the effects of vacuum packaging on the microbial growth in cold and hot smoked rainbow trout during chilled storage.

2 LITERATURE REVIEW

Man has used smoke for preservation and preparation of food for thousands of years (Krasemann 2004). Several methods are available for smoking and different smoked products have been developed in various parts of the world in relation to the properties of the locally available raw materials and the general level of technology (Olley *et al.* 1988).

Fish with high oil content usually make the best smoked product. Today smoking for preservation is common in less developed countries. Smoking for texture and flavour is also popular in developed countries where an integrated logistical infrastructure for the efficient transportation of perishables is in place (Krasemann 2004).

The process of smoking includes different preservative steps such as salting, drying and smoking and, in the case of hot smoking, application of heat.

2.1 Trimming

Fish for smoking must be of high quality, fresh and free from disease. The first step, preparation of raw material for smoking, depends on the species and size of the fish and on the intended form of the product. Trimming may include filleting, splitting or chunking (Krasemann 2004).

2.2 Brining

Salting involves brining and dry salting and sometimes the injection of salt. The concentration of brine and time of brining depends on the type of product and the amount of salt that is desired for the final product, various salt additions are used for smoked products ranging from 2% to 20%. Nowadays three types of cure are mainly used, light, medium and heavy (Razavi 1994). The moisture and salt content in the different types is shown in Table 1. The amount of salt in the final product is often about 2-5%. Salt for sea trout, mackerel and salmon may go up to 5%. A minimum of 3.5g of salt per 100 g of tissue fluid is recommended but salt content in tissue fluid varies in relation to fat content (Table 2).

Table 1: Moisture and salt content in different types of cure (Razavi 1994).

Type of cure	**Salt per 100 kg of fish (kg)**	**Loss in weight (%)**	**Moisture content of fish (%)**	**Salt content (wet basis) (%)**	**Curing time at 18°C (8 days)**
Light	8 10	16 18	74 72	4 6	2 2
Medium	12 14	20 22	70 64	8 9	3 5
Heavy	16 30	26 30	63 57.5	10 20	8 21

Table 2: Salt content of tissue fluid in relation to fat content of raw material (TRS 1989).

Fat content%	Salt content(g salt per 100 g tissue fluid)
0	2.5
10	2.2
20	1.9
30	1.6

2.3 Drying

Drying is a very important part of the smoking process. Usually the temperature for drying is between 20-26°C. Air flow to the product must have specified characteristics (speed, volume, temperature) in relation to the difference of temperature between the air and the products. If wetness or time for drying increases, products will lose their desired quality (Razavi 1994). Speed of drying is influenced by a number of factors, the speed of the air flow, the moisture content of the fish, the temperature and moisture content in the smoke and most importantly, the relative humidity (RH) in the surrounding air. Humidity can affect the speed of drying by limiting the absorption of water by the air. RH is usually about 65% at 30°C, which are very good conditions for drying.

An RH of less than 65% may cause hardening of the product and an RH higher than 65% will prevent effective drying (Krasemann 2004).

2.4 Hot and cold smoking

After salting, a combination of drying and smoking with a temperature of about 20-30°C for cold smoking and between 70-80°C for hot smoking is used for smoked products (FRW 2004).

2.4.1 Cold smoking in Europe

Smoking is a very old preservation technique, which is still used today. Smoking lends a particular flavour to food. Before being smoked, fish is first salted and then dried. Smoking takes place at 30°C for three to six hours (Hafsteinsson 1999). However, the length of the smoking step in Iceland (15 minutes) is short in comparison to European methods. Cold smoke yields milder and more natural tasting products, which are still raw with a deep colour. These products are favoured by Eastern Europeans and Scandinavians. Cold smoking is possible for fish such as salmon (CEJA 2005).

Cold smoked fish is categorized as “lightly preserved” in Europe. This group includes fish products preserved in low levels of salt and heat and they are often vacuum packed and stored at <5°C or frozen. These products are typically ready-to-eat(IFT 2001).

2.4.2 Hot smoking

Traditionally in Iran, fresh fish is gutted and in order to decrease the growth of bacteria and to improve the smoking process the fish is washed in chloral water (25-

50 ppm). For salting, salty water with a viscosity of 0.1 to 0.2 kg Nacl/l is usually used. Sometimes sodium nitrite is used for preserving the colour of the fish. Drying is performed at 20°C and the fish is smoked at 70-80°C for six to eight hours. In the hot smoking method, the temperature is very important and most products have a wetness of about 60%. The air flow is also important, and must be controlled because it can affect the distribution of temperature and moisture (Razavi 1994).

2.5 Quality and safety issues

Post process changes in smoked fish can result from the presence and activity of micro organisms and chemical reactions.

2.5.1 Safety of smoked fish

Fish can carry bacteria that can cause infection or intoxication in humans. These bacteria can either be a part of the indigenous flora or their presence is a result of contamination. The indigenous flora includes bacteria such as *Plesiomonas shigelloides, Vibrio parahaemolyticus V. cholerae V. vulnificus, Cl.botulinum and Listeria monocytogenes*. Regarding the indigenous flora, processing for safety is the key factor to ensuring the safety of the smoked product.

Hygiene is important to prevent contamination and if contamination occurs processing for safety is applied. Microbial contamination of smoked fish include: *Staphylococcus aureus* and *Listeria monocytogenes* and from the animal/human reservoir *Salmonella, Shigella and E.coli* (Huss 2003).

This contamination has normally been associated with faecal contamination or pollution of natural waters or water environments, where these microorganisms may survive for a long time or through direct contamination of products during processing (Liston 1991).

2.5.2 Microbial spoilage of smoked fish

Microbial spoilage results from the activity of bacteria with spoilage potential (i.e. the ability to produce off-odours). The spoilage activity is affected by several parameters. These parameters can be grouped into four categorize.

a- Intrinsic factors such as water activity, acidity, redox potential, available nutrients and natural and microbial substances that are physical, chemical and structural properties inherent in the food.

b- Extrinsic factors like temperature, humidity and atmosphere composition that are factors in the environment in which the food is stored.

c- Modes of processing and preservation and physical or chemical treatments often result in changes in the characteristics of a food product which influences the microflora associated with the product.

d- Implicit parameters are the result of the development of a micro organism which may have a synergistic or antagonistic effect on the microbial activity of other micro organisms present in the product (Jos 1996).

However, for high quality products, storage at a low temperature is required (Feldhusen 2000). During storage, psychotropic lactic acid bacteria (LAB) usually become the dominant bacterial group in smoked products. The LAB for foods belongs to the genera of *Carnobacterium, Enterococcus*, *Lactobacillus, Lactococcus, Leuconostoc*, *Paralactobacillus, Pediococcus and Streptococcus* (Lyhs 2002).

LAB are not considered to belong to aquatic environments but certain species (i.e. *Carnobacterium, Vagococcus, Lactobacillus, Enterococcus, Lactococcus)* have been found in fresh water fish and their environment.

A few studies dealing with vacuum packaged hot smoked fish concentrate on the influence of processing on the microbiological quality. *Carnobacterium* domination has been reported in vacuum-packaged hot smoked rainbow trout at the end of the storage period at 8 and 20°C. While in vacuum packaged cold smoked fish products, the spoilage microflora has usually been dominated by LAB at varying levels including the genera of *Lactobacillus, Leuconostoc, Lactococcus* and *Carnobacterium* (Lyhs 2002).

2.5.3 Chemical spoilage

Chemical spoilage is the result of enzyme action or non-enzymatic reactions like oxidation and the Maillard reaction. The main contributions of chemical spoilage to food are flavour and colour changes due to oxidation, lipolysis and heat. These changes may be induced by light, metal ions or excessive heat during processing or storage (Huss 1994).

3 MATERIALS AND METHODS

3.1 Raw material

Rainbow trout (1 kg mean rounded weight) was supplied from Nordurlax, Laxamyri commercial farm close to Husavik in the north part of Iceland on the 5th December 2005. The fish were slaughtered in the morning and put in boxes with ice and was sent to the Norðlenska Company in Akureyri, Iceland where the smoking took place.

Samples from fresh fish were collected through the whole process and during each step such as salting, drying, and smoke processing. These samples were analysed for chemical parameters. Microbial samples were collected from fresh fish, after smoking and two weeks after smoking.

All samples for microbial analysis were transferred to the laboratory in sterile bags; all fish were tagged for the experiments and also for measurement of weight.

The samples for the microbial analysis and TVB were placed in a refrigerator at 6°C. This temperature was chosen to speed up the post process changes and is not to be recommended. All samples for chemical analysis were frozen immediately and stored at -18^{o}C until the measurement could take place.

3.2 Process of hot and cold smoking

Both smoking methods were implemented at the same time. Table 3 shows the different steps of the hot and cold smoking processes and the schemes for cold and hot smoke fish processing are shown in Figures 1 and 2.

Table 3: Processing steps for hot and cold smoking**.**

Method	**Steps**
Cold smoking	-Filleting -Dry salting (15 hours) -Rinsing ,storage (5 hoursat 2^{o}C) -Drying (17 hours at 20-23°C) -Drying with air blow (45 min at 20^{o}C) -Smoking (15 min at 20-23°C) -Storage (at 6^{o}C)
Hot smoking	-Gutting -Washing with chloral water (25-50 ppm) -Brine salting (24 hours,0.134 kg pure salt and 0.066 kg salt with nitrite sodium in 1 lit) -Drying (76 hours at 20^{o}C) -Drying with air blow (8 hours at 20^{o}C) -Smoking (6 hours at 70^{o}C) -Storage (at 6^{o}C)

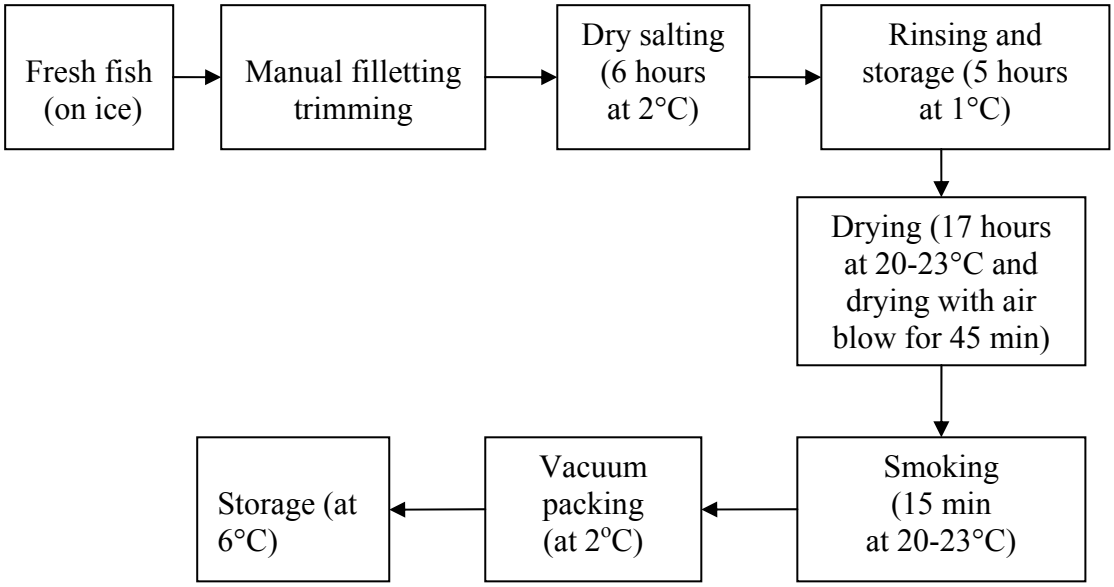


Figure 1: A scheme for the cold smoking of rainbow trout.

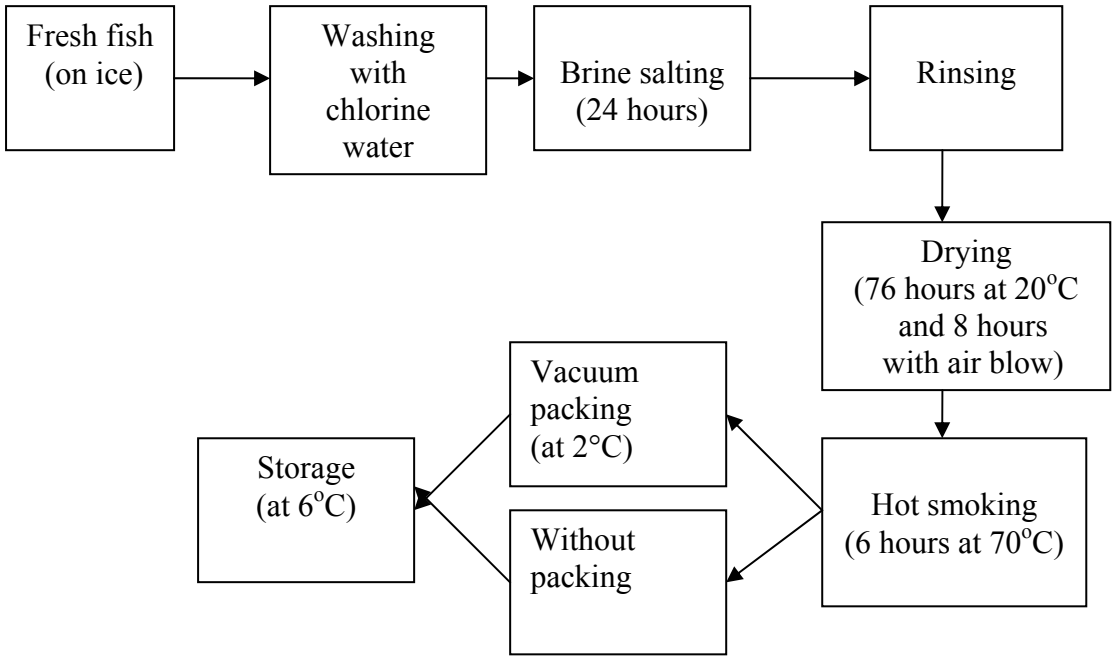


Figure 2 : A scheme for the hot smoking of rainbow trout.

Different steps of the hot and cold smoking processes are shown in Figures 3 to 8.

Figure 3: Raw rainbow trout.

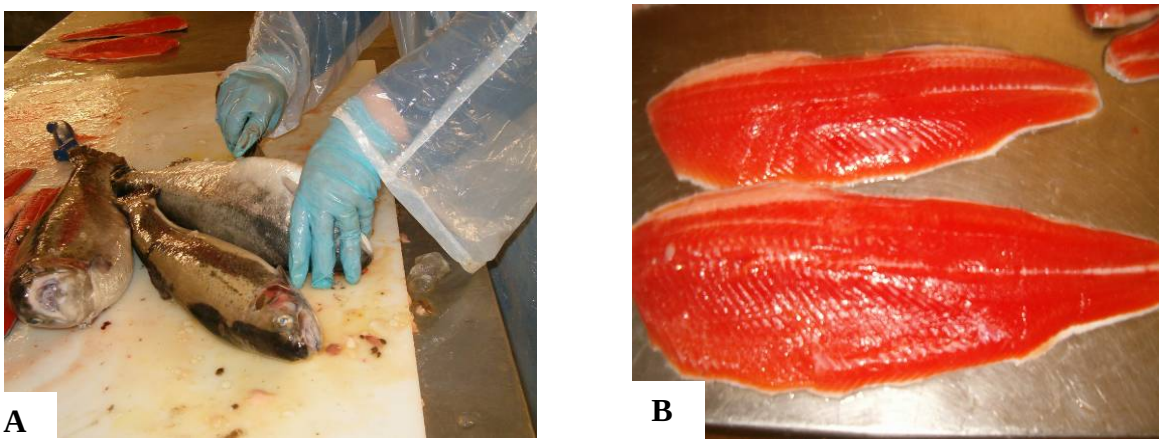


Figure 4: Fish after gutting for hot smoking (A). Fish after filleting for cold smoking (B).

C

D

Figure 5: Brine salting for hot smoking (C). Dry salting for cold smoking (D).

E

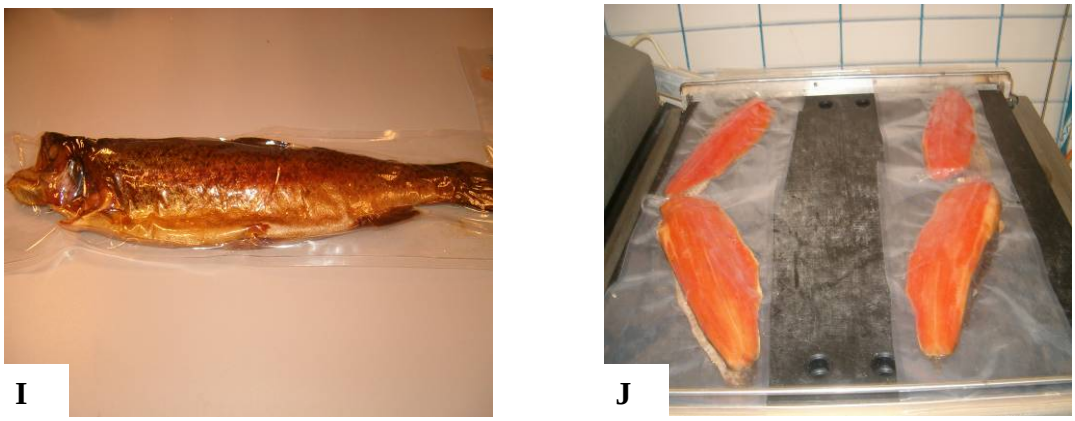
F

Figure 6: Whole fish hanging in the hot smoking chamber (E). Trout fillets on racks for cold smoking (F).

G

H

Figure 7: Trout after hot smoking (G). Trout fillet after cold smoking (H).



I J

Figure 8: Hot (I) and cold (J) smoked trout in vacuum packing.

3.3 Sampling

Samples were taken randomly during processing for chemical and microbial analysis, until two weeks after processing and storage. Samples for microbial examination were divided in three parts:

1- Cold smoked products vacuum packed
2- Hot smoked products vacuum packed
3- Hot smoked products not vacuum packed

Different steps for sampling and the numbers of samples for chemical and microbial analysis are shown in Tables 4 and 5. For each chemical and microbial analysis, two samples were selected for each processing step.

Samples for chemical analysis were collected from rainbow trout at different steps of the smoking process and samples for microbial analysis were collected before and after smoking and after two weeks of storage.

Table 4 : Number of samples for chemical experiments in each step of processing for cold and hot smoking.

Processing steps for chemical sampling	**Cold smoking**	**Hot smoking**
Raw fish	3 fillets of fish	3 whole fish
Salting	2 samples at 3 hours of salting 2 samples at 15 hours of salting	2 samples at 3 hours of salting 2 samples at 15 hours of salting 2 samples at 24 hours of salting
Rinsing and storage	2 samples after keeping (at 1°C) for 5 hours in refrigerator	
Drying	2 samples after 17 hours drying at 20-23°C	2 samples after 11 hours of drying at 20-23°C and 6 hours with air blow at 20°C
	2 samples after drying for 45 min with air blow	2 samples after 84 hours drying
Smoking	2 samples after smoking	2 samples at 2 hours of smoking 2 samples at 6 hours of smoking
2 weeks after processing	2 samples after storage	2 samples after storage
Total number of samples	17 samples	19 samples

Table 5: Number of samples for microbial experiments in each step of processing for cold and hot smoking.

Processing steps for microbial sampling	**Cold smoking**	**Hot smoking with packing**	**Hot smoking without packing**
Raw fish	3 fillets of fish	3 whole fish	
After processing	2 samples	2 samples	2 samples
1 week after storage at 6°C	2 samples	2 samples	2 samples
2 weeks after storage at 6°C	2 samples	2 samples	2 samples
Total number of samples	9 samples	9 samples	6 samples

3.4 Chemical analysis

3.4.1 Chemical analyses were performed according to procedures in the Icelandic Fisheries Laboratory and were as follows:

3.4.2 TVB-N determination (Malle and Poumeyrol 1989)

For measurements of TVB, 100g of sample was minced and then weighed and 200 ml of 7.5% aq tricholoroacetic acid was added, mixed for 1 minute, in a Waring blender, and then filtrated to make an extract. Next, 25 ml of filtrate was transferred into a distillation flask and 6 ml of 10% NaoH was added, and distilled for 4 minutes. Distillate was gathered into 10 ml of 4% boric acid with methyl red and bromocresol green. Distillate was titrated with 0.025N H_2SO_4, until a pink colour appeared in the solution.

Calculation (mgN/100g): $\frac{14 \text{ mg/mol} * a * b * 300}{25 \text{ ml}}$

a: ml of sulphuric acid
b: Normality of sulphuric acid

3.4.3 Salt determination

Salt content was determined by the volumetric method of Volhard (AOAC 937.09 1990). 5 g of sample were weighed into a conical flask and 200 ml of distilled water added. The flask was placed on an electric shaker for 45 minutes. Then 20 ml of the solution was poured into an Erlenmeyer flask and the chloride ions precipitated by adding 10 ml of 0.1 N $AgNO_3$. The excess $AgNO_3$ was titrated with 0.1 N NH_4SCN solution, a ferric indicator ($FeNH_4(SO_4)_2$*$12H_2O$ in diluted HNO_3) was added for determination of the end point, when a fair red brownish colour appeared. The salt content was calculated as a percentage of the sample.

$$\text{MmolCl/l} = \frac{(B-S)*N_{NH_4SCN}*1000}{\text{Volume of sample}}$$

B= ml titrant for blank
S= ml titrant for sample

$$\%\text{Nacl} = \frac{\text{mmolCl/l}*0.2\text{L}*58{,}5\text{g/mol}*0{,}001*100}{\text{gr sample}}$$

3.4.4 Protein determination (Kjeldahl nitrogen determination)

For protein measurement, 0.2-0.5 g of minced fish were accurately weighed into a Kjeldahl digestion flask (1009 digester tecator) (AOAC 955.04 1990) with two catalyst tablets (each containing 0.4 g $CuSO_4$ and 3.5 g K_2SO_4). Next, 6 ml of concentrated sulphuric acid were added and heated for four hours at 420^{o}C and after digestion, the flask was cooled and 20 ml of distilled water was added to the flask, and the flask was placed in the Kjeldahl Autosampler system (Kjeltec system 1002 distilling unit). After adding 20 ml of 40% sodium hydroxide, distillation of ammonia was started in 25 ml 1% boric acid. The nitrogen content was determined by titration with a 0.1 N HCl.

The protein content was calculated as:

$$\%\text{ Nitrogen} = \frac{\text{ml } H_2SO_4 * N_{H_2SO_4} * 14\text{ g/mol} * 100}{\text{g (mass of sample)} * 1000}$$

$$\%\text{ Protein} = \%\text{ Nitrogen} * 6.25$$

3.4.5 Fat determination

A dry soxlet flask was accurately weighed; 5g of the sample was dried at 100°C for four hours and placed in a Whatman cellulose extraction thimble, which was placed in a soxlet extraction tube. Fat was extracted in the Soxtec system with petroleum ether for six hours. Then the ether was separated from the oil with a Buchi Rotavapor R-114 waterbath. The Soxlet flask was placed in an oven (100°C) for 20 minutes and was cooled in a desiccator. Then the flask was weighed accurately (AOAC1989).

$$\text{Fat \%} = \frac{(\text{weight of flask with fat} - \text{weight of flask})*100}{\text{Weight of the sample}}$$

3.4.6 Ash determination

10g of sample were accurately weighed in a crucible. Preliminary heating was carried out to allow smoking off from fat without burning. The sample was then placed in a furnace at 500-550^{o}C until a white ash appeared (AOAC 938.08-1990).

$$\text{Ash content} = w\ \frac{\text{(burned sample + dish)-w (dish)}}{\text{W (sample)}}$$

3.4.7 Moisture determination

5 g of the sample were accurately weighed into a porcelain dish and mixed with sand and blended with a glass rod. Moisture content (g/100g) was calculated as the loss in weight, after drying at 103°C for four hours (ISO 1999).

$$\text{Moisture content} = 1 - \frac{(W_{Ds}+W_{Dish}) - W_{Dish}}{W_{Sample}} * 100$$

$W_{Ds=}$ weight of dried sample
$W_{Dish=}$ weight of (dish+sand+glass rod)

3.5 Microbial analysis

For the microbial counts, 25g of minced sample and 225g of sterile Butterfields buffer were mixed in a stomacher for one minute. Tenfold dilutions series were made in the same buffer.

3.5.1 Total plate count by pour plate method

1 ml of each of the sample dilutions was transferred with pipettes onto Petri plates. Melted Plate Count Agar (DIFCO) at 45°C was poured on the plates and the content mixed. Plates were incubated at 30°C for 48 hours. Colonies were usually counted over light with a double magnification. In a Quebec colony, counter colonies could also be counted by inverting the plates and marking each colony with a marker (APHA 1992).

3.5.2 Lactic acid bacteria

1 ml of each of the sample dilutions was transferred with pipettes onto Petri plates. MRS agar (DIFCO) may be used for the cultivation of the whole group of lactic acid bacteria. Reading the results should be performed after a defined incubation time (30°C+22°C mesophilic- psychotrophic:2+1 days and 25°C psychotropic from meat:3 days).

All well grown colonies are considered as lactic acid bacteria (IJFM 1987).

3.5.3 Total coliform and faecal coliform counts by the most probable method (MPN)

Lauryl sulphate tryptose (LST) broth (DIFCO) was used as a pre-enrichment media. A set of three tubes was used for each dilution. The first three tubes contained double concentration of LST, 10 ml of a 1/10 dilution were transferred into the first three tubes, 1 ml of the 1/10 dilution into the second set and 1 ml of the 1/100 dilution was

transferred in the third set. After inoculation the tubes were incubated at 35°C for (24/48) hours. One loopful from the positive tubes (gas formation) was transferred into tubes of Ec medium and BGLB/Brilliant green lactose bile broth media (DIFCO). The EC tubes were incubated at 44.5°C in a water bath for 24 hours, and the BGLB tubes were incubated at 35°C for 48 hours. Gas production in Ec medium confirms faecal coliforms, and gas production in the BGLB media confirms total coliforms. The number of positive tubes within each dilution was recorded and comparable triplet was found in MPN tables. (APHA 1992).

3.6 Statistical analysis

Duncan tests and t-tests were performed by the ANOVA method in the STATISTICA program at P value less than 0.05 in order to consider significant differences between data for the cold and hot smoking processes.

4 RESULTS

4.1 Chemical analysis

Significant water loss was observed in both cold and hot smoked products. Because of this, the percentages of protein, fat and salt increased during the smoking process. The initial nutrition value of the raw material and the results for the products after two weeks of storage are shown in Table 6. The results for raw fish (fillets and whole fish) are very similar. However, after processing the percentages of protein and salt in hot smoked products are higher than in cold smoked products but percentages of fat in cold and hot smoked products are not significantly different.

Table 6: Nutritional value of raw material and smoked rainbow trout.

Experiments	Raw material		Products after 2 weeks	
Result in %	Hot smoking	Cold smoking	Hot smoking	Cold smoking
Water	74.05	74.48	63.53	67.03
Protein	22.01	20.88	27.22	23.48
Fat	3.74	3.87	6.35	6.47
Salt	0.52	0.46	2.06	1.31

4.1.1 Weight

Weight decreased during processing and after drying for both of cold and hot smoked products. All of the samples showed a considerable decrease in weight after processing varying from 35.3% for hot smoked products to 16.9% for cold smoked products. The decrease of weight during hot and cold smoking is shown in Figure 9 below (Table 9 in Appendix).

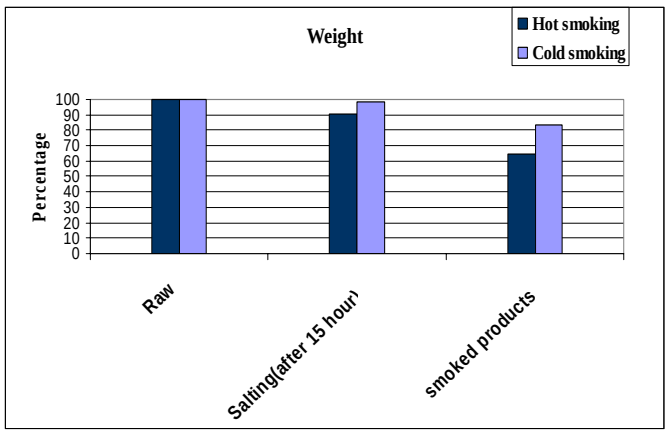


Figure 9: Reduction in the weight of fish during hot and cold smoking.

4.1.2 Water content

The initial water content was 74% and it decreased during brining and dry salting for both cold and hot smoking. The water content also decreased rapidly during drying and smoking for hot smoked products and is shown in Figures 10 and 11 (Table 10 in Appendix). The reduction of water content is 10% for cold smoking and 14.2% for hot smoking.

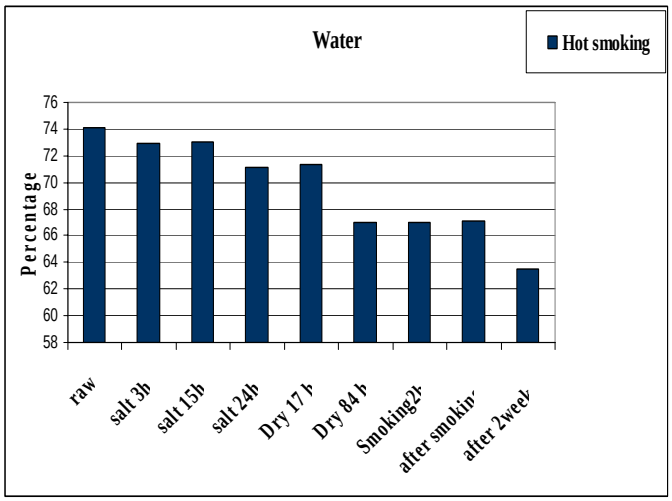


Figure 10: Changes in percentage of water content during the hot smoking process.

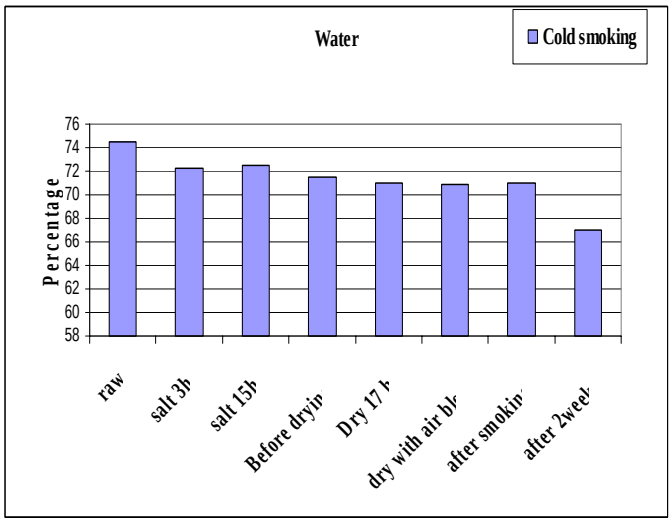


Figure 11: Changes in percentage of water content during the cold smoking process.

4.1.3 Protein content

The percentage of protein increased during drying and smoking, which corresponds with the decreasing percentage of water. Increasing percentages of protein are shown in Figures 12 and 13 (Table 13 in Appendix). The percentage of protein increased from 22% to 27.2% for hot smoking and from 20.9% to 23.5% for cold smoking.

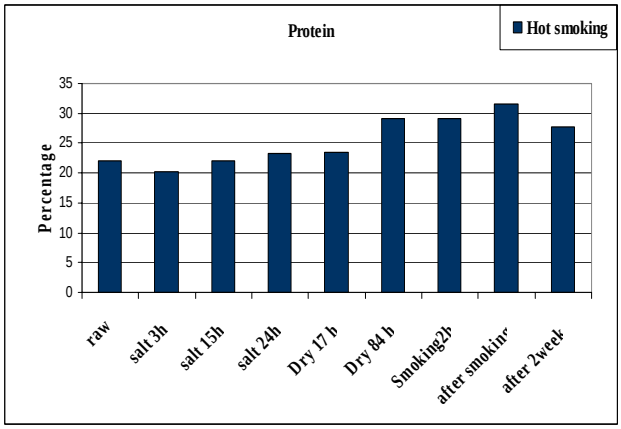


Figure 12: Changes in percentage of protein during the hot smoking process.

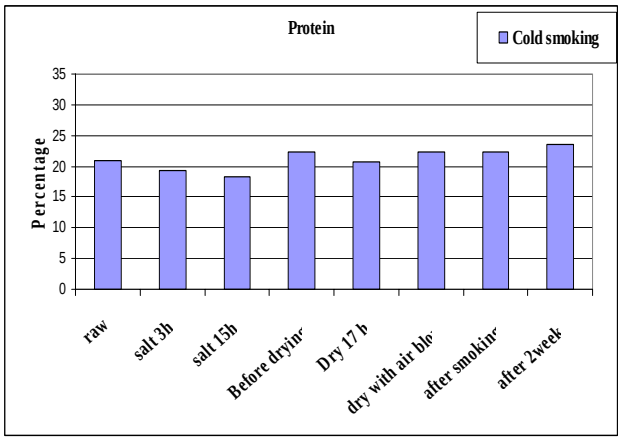


Figure 13: Changes in percentage of protein during the cold smoking process.

The decrease of water and increase of protein during hot and cold smoking are shown in Figures 14 and 15 (Tables 10 and 13 in Appendix).

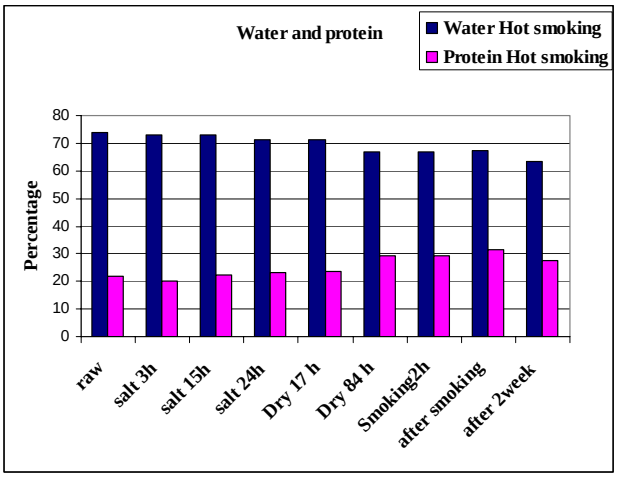


Figure 14: Changes in percentage of water and protein content during the hot smoking process.

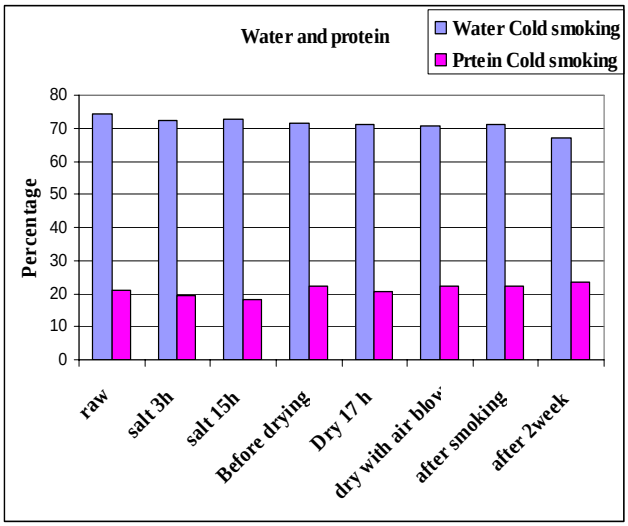


Figure 15: Changes in percentage of water and protein content during the cold smoking process.

4.1.4 Ash

The percentage of ash did not change considerably for cold or hot smoked products. Changes in the percentage of ash are shown in Figures 16 and 17 (Table 11 in Appendix)

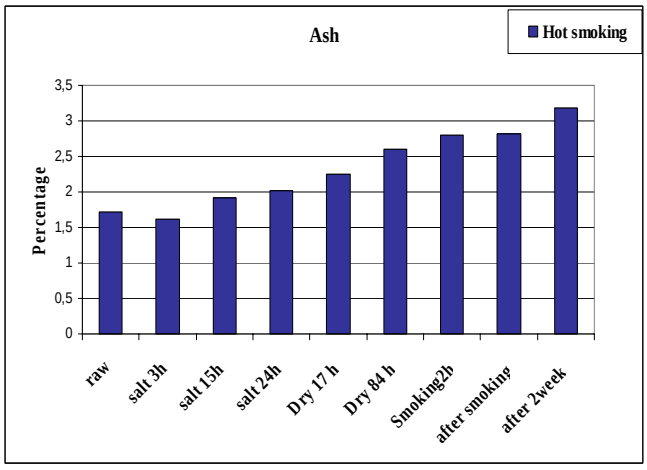


Figure 16: Changes in the percentage of ash during the hot smoking process.

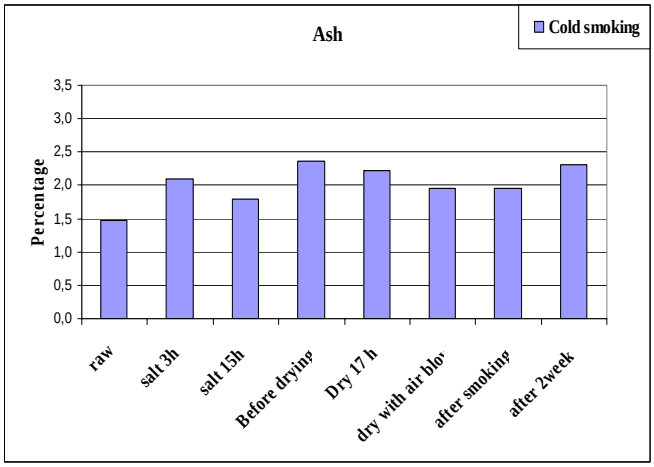


Figure 17: Changes in the percentage of ash during the cold smoking process.

4.1.5 Salt content

The percentage of salt increased for both hot and cold smoking. The increase of salt during drying for hot smoking was more rapid than for cold smoking. All samples showed increases in the percentage of salt during processing of 150% for hot smoked and 80% for cold smoked products. Changes in the percentage of salt are shown in Figures 18 and 19 (Table12 in Appendix)

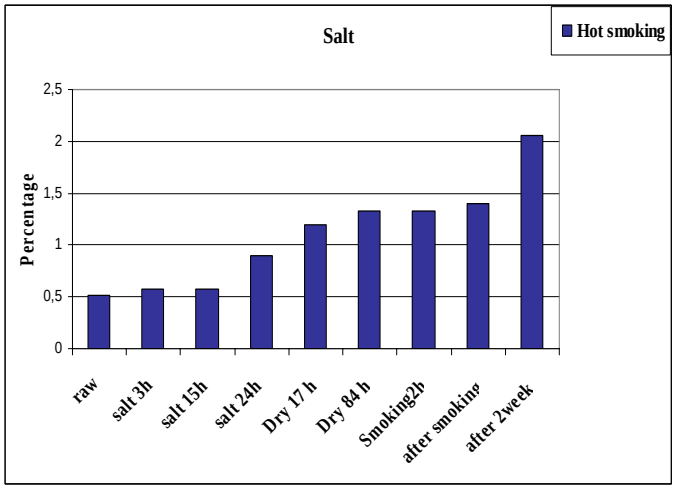


Figure 18: Changes in the percentage of salt during the hot smoking process.

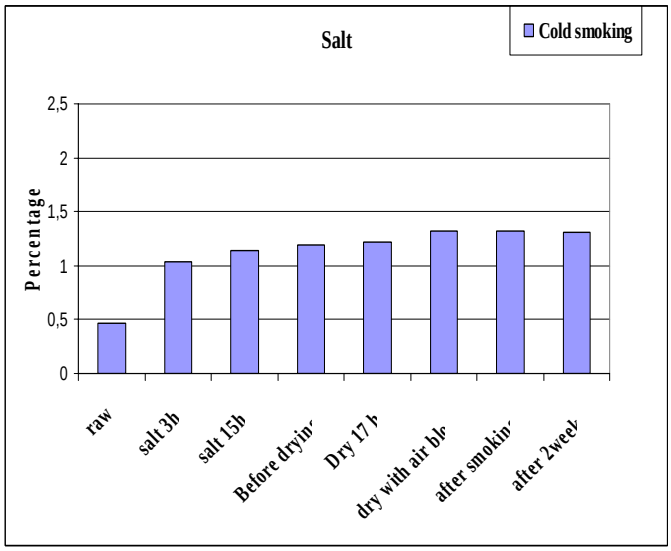


Figure 19: Changes in the percentage of salt during the cold smoking process.

4.1.6 Fat content

The percentage of fat increased during salting for hot smoking but decreased during the drying and smoking steps. The fat content of cold smoked fish increased during salting but didn't change after the salting procedure. The percentage of fat for hot smoking increased from 69.5% and to 67% for cold smoking. The changes in fat content are shown in Figures 20 and 21 (Table 14 in Appendix).

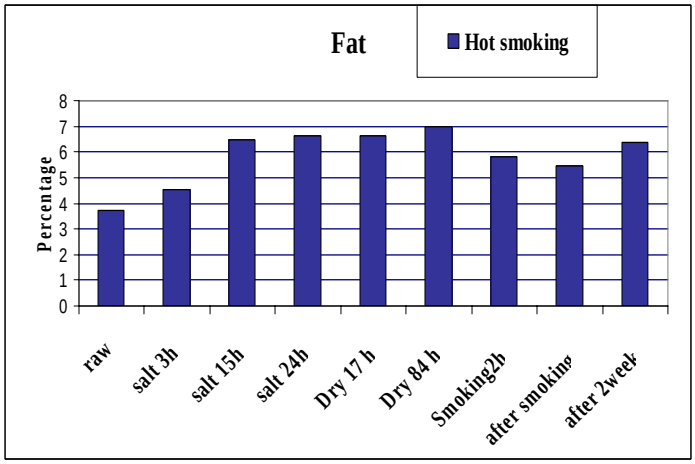


Figure 20: Changes in the percentage of fat during the hot smoking process.

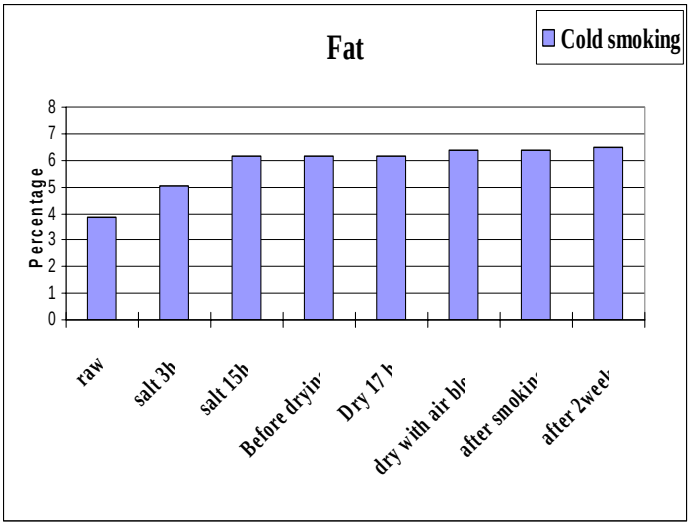


Figure 21: Changes in the percentage of fat during the cold smoking process.

4.1.7 Total volatile basic amine

The amount of TVB for all of the products increased after processing and storage at 6°C (Figure 22 and Table 15 in Appendix). The change of TVB for: cold smoking was from 24.33 to 45.36 mgN/100 g; for hot smoked products with vacuum packing the change was from 20.34 to 35.165 mgN /100 g; and for hot smoked products without packing the change was from 20.34 in the raw fish to 31.3 mgN/100 g in the smoked products .The increase of TVB for the cold smoked packed product after two weeks of storage is higher than packed and unpacked hot smoked products.

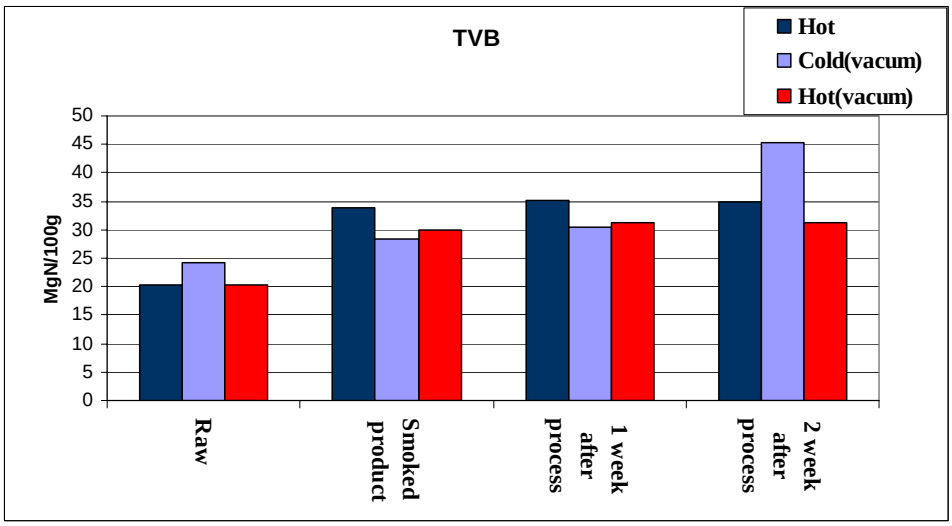


Figure 22: Changes in TVB for hot and cold smoked rainbow trout.

4.2 Microbial results

4.2.1 Total plate count

The results of microbial analysis indicated that cold smoked products are more sensitive to microbial spoilage than hot smoked products, both with and without packaging. The total count of bacteria for cold smoked products after one and two weeks of storage at 6°C were between 10^6 and10^7.. Log CFU/g of the total count for the two methods is shown in Figure 23 (Table16 in Appendix).

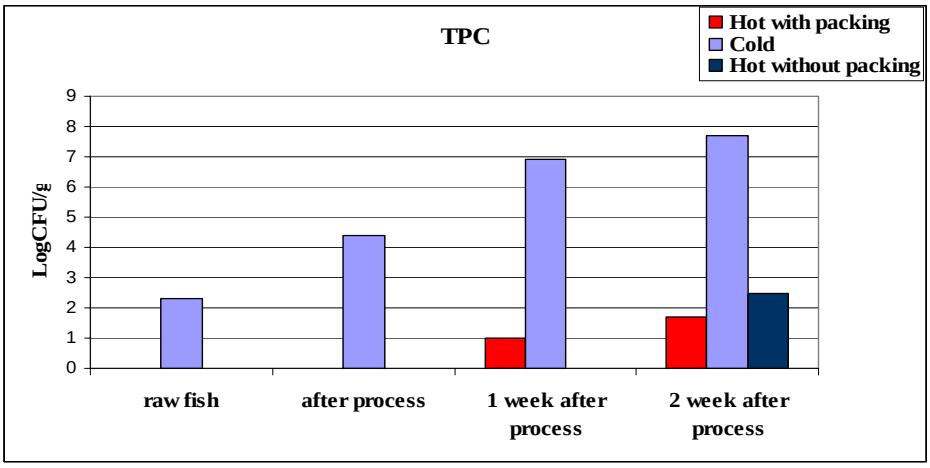


Figure 23: Changes in the total plate count for the two methods of smoking.

4.2.2 Lactic acid bacteria

Lactic acid bacteria were found in cold smoked products ranging from 10^6 in the first week of storage to 10^7 CFU/g after two weeks of storage at 6°C. For hot smoked products with and without packing, the amount of CFU/g did not change considerably. Differences in the logCFU/g of LAB between hot and cold smoked products are shown in Figure 24 (Table17 in Appendix).

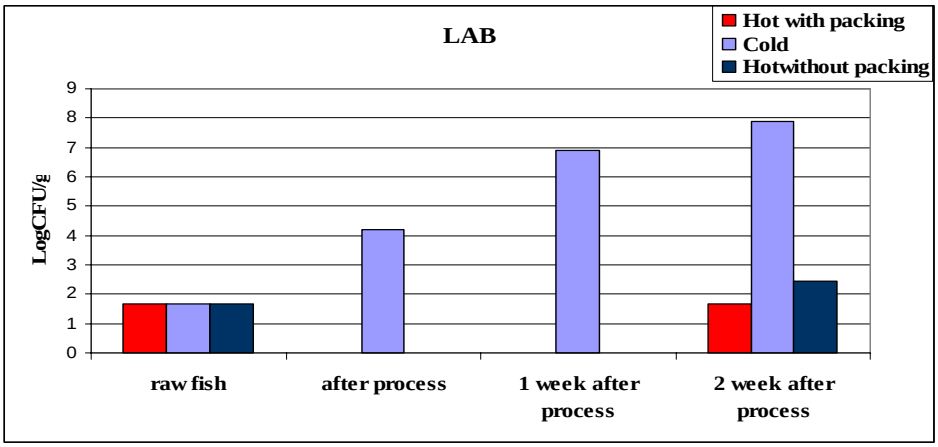


Figure 24: Changes in lactic acid bacteria for the two methods of smoking.

4.2.3 Total and faecal coliform

The results indicated that no total or faecal coliform was found in raw fish, after processing and after one week of storage at 6°C. MPN/g of total and faecal coliform is shown in Table 7.

Table 7: MPN/g of total and faecal coliform for hot and cold smoked rainbow trout.

Line processing	MPN/g Total coliform	MPN/g Faecal coliform
Whole fish	<0.3	<0.3
Fillet of fish	<0.3	<0.3
Hot smoked products with packing	<0.3	<0.3
Hot smoked products without Packing	<0.3	<0.3
Cold smoked products	<0.3	<0.3
Hot smoked products with packing (after 1 week)	<0.3	<0.3
Hot smoked products without packing (after 1 week)	<0.3	<0.3
Cold smoked products (after1 week)	<0.3	<0.3
Hot smoked products with packing (after 2 weeks)	<0.3	<0.3
Hot smoked products without packing after 2 weeks)	<0.3	<0.3
Cold smoked products (after2 weeks)	7.43	<0.3

4.3 Statistical analysis

Significant differences were detected with the t-test and the Duncan test ($\alpha = 0.05$) using the ANOVA method in the STATISTICA programme with the P value less than 0.05, from raw materials and during processing for protein-fat-moisture-salt-ash and after processing for total count, lactic acid bacteria and TVB.

Statistical significance was found between various components of the experiment (Table 8).

Ash content changed significantly during the drying phase of the hot smoking. Water and protein also changed significantly during the drying and smoking phase of the hot smoking method. Salt content changed in both methods during the salting and drying phases.

Total count, LAB and TVB were only significantly different at the end of the two weeks of storage for cold smoking.

Fat was the only test component that did not show significant changes during processing for both methods.

Table 8: Results of statistical analysis for hot and cold smoking.
Duncan test by the ANOVA method.

Experiment	**Method of smoking**	**Step of process with significance difference**
Ash	Hot smoking	During drying
Protein	Hot smoking	During drying and smoking
Fat	---------	----------
Salt	Hot smoking Cold smoking	During salting, drying and smoking During salting
TVB	Cold smoking	After 2 weeks of storage
Water	Hot smoking	During drying and smoking
Total count	Cold smoking	After 2 weeks of storage
Lactic acid bacteria	Cold smoking	After 2 weeks of storage

5 DISCUSSION

The purpose of this study was to compare nutritional and chemical changes during hot and cold smoking of rainbow trout. The results show that during the smoking process, changes in the chemical contents of the fish occurred mainly in the water content, salt, protein and ash. Change in quality during storage at 6°C measured by TVB and bacterial count showed spoilage.

The weight of fish decreased rapidly for both methods of smoking, which can be explained by losses of water. Water content decreased rapidly in all of the samples during salting, but after the salting phase the rate of reduction decreased for cold smoking. The water loss from the muscle was greater for hot smoking than cold smoking because of the long period of salting and drying for hot smoking.

The salt content in the fish increased rapidly during the first hours of salting especially for cold smoking and salt content continued to rise during the last 10 hours of salting and during drying. The difference can be explained by the form of fish that was used for hot and cold smoking (fillet for cold and whole fish for hot smoking). As surrounding brine has much higher salt content than fish muscle, the salt from the brine penetrates the fish muscle until the fish muscle and the surrounding brine has reached equilibrium (Munasinghe 1999).

The protein percentage decreased during the first hours of salting, probably due to leaching of water soluble proteins such as myogen (an albumin type protein) and salt soluble fractions, myosin (a globulin). Myosin constitutes about 75% to 80% of the total protein (Munasinghe 1999). After the salting step, the ratio of protein increased especially for hot smoking but the protein ratio for cold smoking was more stable during drying and smoking than hot smoking.

No statistical difference was found between the two methods in terms of changes in fat content. All changes observed were due to water losses during the salting phase. An obvious sign of spoilage is the detection of off-odours and off flavours, gas production and changes in texture. The development of these spoilage conditions in fish products are due to a combination of microbiological, chemical and autolytic activities. The main spoilage of smoked fish is due to bacterial growth.

Hot smoked products receive heat treatment during processing and may also receive additional heat treatment before consumption.

There are different reasons for the increase of the total count and lactic acid bacteria in cold smoked products:

1- The temperature in the cold smoking process is lower than in hot smoking and isn’t sufficient heat treatment for bacterial growth.
2- The coliforms bacteria are inactivated during heating of hot smoking but the temperature in cold smoking isn’t high enough.
3- Improper cleaning can lead to high bacterial numbers.

In this study the total plate count in rainbow trout was found to be low (between 0 and 200) for fillets and whole fish before processing, but increased steadily during storage at 6°C for cold smoked fish.

An increase of TVB in both methods of smoking and during storage was most likely caused by an autolytic process which produces volatile amine compounds and bacterial spoilage. The influence of storage temperature (6°C) was high for TVB, and for all of the products TVB was increased after processing and during storage at 6°C. TVB for cold smoked products was higher than hot smoked products after two weeks. According to statistical results there are significant differences of TVB between the two methods after storage at 6°C.

The microbiological growth rate in cold smoked products is higher than in the hot smoked products. Bacteria which have been found responsible for spoilage in sterile cold smoked salmon stored in vacuum packs at 6°C were *Lactobacillus sakei, L.farciminis and Brochothrix thermosphacta* which produced sulphurous acidic and rancid off- odours (Dondero *et al.* 2004). Microbiological growth rates in the hot smoked products without packing showed similar trends as packed products during storage but more studies are needed.

The initial total coliform and faecal coliform was < 0.3MPN/mg and one week after processing no changes were observed for all of the products but after two weeks of storage some increases of TOC were seen for cold smoked products.

Lactic acid bacteria in cold smoked products were significantly higher than hot smoked products. Results indicate that cold smoked products are more sensitive than hot smoked products which is related to the temperature of smoking.

Lactic acid bacteria dominated the microflora, throughout the storage period. There are no reported observations on the lactic acid bacteria ability to produce TMA. There is no correlation between shelf life and the LAB count or any other bacterial number (Dondero *et al.* 2004). They may be found in high numbers before the product is spoiled.

6 CONCLUSION

This study shows that rainbow trout is a good raw material for Iranian traditional hot smoking. The nutritional quality in hot smoked products is good and the percentage of protein is higher than in cold smoked products.

The results indicate that 6°C is too high temperature for storage of smoked rainbow trout, and to have a longer shelf life a lower temperature than 6°C is recommended.

Comparison of microbiological and chemical parameters of rainbow trout stored at 6°C showed that hot smoked products are still of a good enough quality for human consumption after two weeks and results indicate hot smoked products without packing do not have greater bacterial contamination than hot smoked products with vacuum packing.

The results indicate some difference in chemical parameters during processing and these changes were significant for TVB and protein. Higher amounts of TVB for cold smoked products indicate that after two weeks of storage at 6°C, they aren't suitable for human consumption.

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APPENDIXES

Table 9: Percentage of weight during hot and cold smoking process

Weight	**Hot smoking**	**Cold smoking**
Fish	100	100
Salting(after 15 hour)	90.394	98.6
Smoked product	64.77	83.13

Table 10: Percentage of water during hot and cold smoking process

Processing line	**Hot smoking**	**Cold smoking**
Fish	74.05	74.48
Salting(after 3hour)	72.91	72.3
Salting(after 15 hour)	73	72.56
Salting(after 24 hour)	71.14	------
After salting ,storage in refrigerator at 1°C for 5 hour	-------	71.48
Drying(after 17 hour at ambient temp)	-------	71.02
Drying(after 11 hour at ambient+ 6 hour with air blow)	71.32	-------
Drying(after 17 hour at ambient+45 min with air blow at ambient temp)	**------**	70.9
After drying (84 hour)	68.255	-------
After smoking(2 hour)	67	-------
After smoking(6 hour for hot and 15min for cold smoking)	67.14	70.96
After 2week	63.53	67.03

Table 11: Percentage of ash during hot and cold smoking process

Processing line	**Hot smoking**	**Cold smoking**
Fish	1.71	1.47
Salting(after 3hour)	1.62	2.09
Salting(after 15 hour)	1.915	1.80
Salting(after 24 hour)	2.022	------
After salting ,storage in refrigerator at 1°C for 5 hour	-------	2.37
Drying(after 17 hour at ambient temp)	-------	2.22
Drying(after 11 hour at ambient+ 6 hour with air blow)	2.245	-----
Drying(after 17 hour at ambient+45 min with air blow at ambient temp)	-------	1.96
After drying (84 hour)	2.48	------
After smoking(2 hour)	2.8	**-----**
After smoking(6 hour for hot and 15min for cold smoking)	2.81	1.96
After 2week	3.18	2.305

Table 12: Percentage of salt during hot and cold smoking process

Processing line	**Hot smoking**	**Cold smoking**
Fish	0.515	0.46
Salting(after 3hour)	0.57	1.03
Salting(after 15 hour)	0.58	1.14
Salting(after 24 hour)	0.9	------
After salting ,storage in refrigerator at 1°C for 5 hour	-----	1.19
Drying(after 17 hour at ambient temp)	-----	1.22
Drying(after 11 hour at ambient+ 6 hour with air blow)	1.195	------
Drying(after 17 hour at ambient+45 min with air blow at ambient temp)	-----	1.32
After drying (84 hour)	1.26	-----
After smoking(2 hour)	1.325	-----
After smoking(6 hour for hot and 15min for cold smoking)	1.4	1.32
After 2 week	2.06	1.31

Table 13: Percentage of protein during hot and cold smoking process

Processing line	**Hot smoking**	**Cold smoking**
Fish	22.01	20.88
Salting(after 2hour)	20.295	19.405
Salting(after 15 hour)	22.135	18.32
Salting(after 24 hour)	23.345	------
After salting ,storage in refrigerator at 1°C for 5 hour	-------	22.41
Drying(after 17 hour at ambient temp)	------	20.795
Drying(after 11 hour at ambient+ 6 hour with air blow)	23.405	------
Drying(after 17 hour at ambient+45 min with air blow at ambient temp)	------	22.23
After drying (84 hour)	28.835	------
After smoking(2 hour)	29.095	------
After smoking(6 hour for hot and 15min for cold smoking)	31.6	22.2
After 2week	27.22	23.48

Table 14: Percentage of fat during hot and cold smoking process

Processing line	**Hot smoking**	**Cold smoking**
Fish	3.74	3.87
Salting(after 2hour)	4.52	5.06
Salting(after 15 hour)	6.465	6.18
Salting(after 24 hour)	6.63	-----
After salting ,storage in refrigerator at 1°C for 5 hour	-----	6.18
Drying(after 17 hour at ambient temp)	-----	6.135
Drying(after 11 hour at ambient+ 6 hour with air blow)	6.6	-----
Drying(after 17 hour at ambient+45 min with air blow at ambient temp)	-----	6.39
After drying (84 hour)	6,97	-----
After smoking(2 hour)	5.805	-----
After smoking(6 hour for hot and 15min for cold smoking)	5.465	6.39
After 2week	6.35	6.465

Table 15: mgN/100g of total volatile basic amine for raw fish and after processing and storage at 6°C

Processing line of smoking	**Hot smoked product With vacuum packing**	**Hot smoked product without vacuum packing**	**Cold smoked product with Vacuum packing**
Fish	20.34	20.34	24.33
After processing	29.82	33.81	28.35
1 week after processing	31.305	35.165	30.165
2 week after processing	31.3	35.00	45.36

Table 16: CFU/g of total count for cold and hot smoked product

Step of Sampling	**Cold smoked product**	**Hot smoked product with packing**	**Hot smoked product without packing**
Raw fish	200	0	0
After process	25525	0	0
1 week after process	$7.81*10^6$	0	10
2 week after process	$5.16*10^7$	50	300

Table 17: CFU/g of Lactic acid bacteria for cold and hot smoked product

Step of sampling	**Cold smoked product**	**Hot smoked product with packing**	**Hot smoked product without packing**
Raw fish	50	0	50
After process	$1.6*1o^4$	0	0
1 week after process	$7.85*10^6$	0	0
2 week after process	$7.92*10^7$	50	275

STAT. Duncan test; TVB (new.sta)
GENERAL Probabilities for Post Hoc Tests
MANOVA INTERACTION: 1 x 2

SMOKING		STIME	{1} 20,34000	{2} 33,81000	{3} 35,16500	{4} 35,10000	{5} 20,34000
hot	1	{1}		,032822*	,021567*	,021585*	1,000000
hot	2	{2}	,032822*		,802519	,801258	,031922*
hot	3	{3}	,021567*	,802519		,989974	,021105*
hot	4	{4}	,021585*	,801258	,989974		,021016*
hotv	1	{5}	1,000000	,031922*	,021105*	,021016*	
hotv	2	{6}	,111849	,476551	,355390	,356651	,107809
hotv	3	{7}	,073453	,625943	,490817	,486018	,071752
hotv	4	{8}	,071869	,643644	,496915	,497481	,069538
cold	1	{9}	,464585	,114493	,077722	,078072	,440677
cold	2	{10}	,169408	,340973	,246116	,247527	,162332
cold	3	{11}	,281000	,209068	,145925	,146797	,267773
cold	4	{12}	,000572*	,050279	,061035	,071078	,000552*

SMOKING		STIME	{6} 29,82000	{7} 31,30500	{8} 31,30000	{9} 24,32667	{10} 28,35000
hot	1	{1}	,111849	,073453	,071869	,464585	,169408
hot	2	{2}	,476551	,625943	,643644	,114493	,340973
hot	3	{3}	,355390	,490817	,496915	,077722	,246116
hot	4	{4}	,356651	,486018	,497481	,078072	,247527
hotv	1	{5}	,107809	,071752	,069538	,440677	,162332
hotv	2	{6}		,784056	,772806	,330392	,774295
hotv	3	{7}	,784056		,999330	,232650	,596804
hotv	4	{8}	,772806	,999330		,228220	,587091
cold	1	{9}	,330392	,232650	,228220		,460554
cold	2	{10}	,774295	,596804	,587091	,460554	
cold	3	{11}	,534961	,395142	,388152	,679846	,710406
cold	4	{12}	,014671*	,022164*	,023731*	,002239*	,009006*

SMOKING		STIME	{11} 26,44500	{12} 45,36000
hot	1	{1}	,281000	,000572*
hot	2	{2}	,209068	,050279
hot	3	{3}	,145925	,061035
hot	4	{4}	,146797	,071078
hotv	1	{5}	,267773	,000552*
hotv	2	{6}	,534961	,014671*
hotv	3	{7}	,395142	,022164*
hotv	4	{8}	,388152	,023731*
cold	1	{9}	,679846	,002239*
cold	2	{10}	,710406	,009006*
cold	3	{11}		,004676*
cold	4	{12}	,004676*	

STAT. Duncan test; WATER (new2.sta)
GENERAL Probabilities for Post Hoc Tests
MANOVA INTERACTION: 1 x 2

			{1}	{2}	{3}	{4}	{5}	
SMOKING	STIMES			74,04333	72,91000	73,00000	71,14000	71,31500
hot	1	{1}		,612370	,621053	,235174	,260918	
hot	2	{2}	,612370		,965981	,457882	,498201	
hot	3	{3}	,621053	,965981		,438848	,479459	
hot	4	{4}	,235174	,457882	,438848		,933779	
hot	5	{5}	,260918	,498201	,479459	,933779		
hot	6	{6}	,026794*	,066843	,063079	,225917	,205526	
hot	7	{7}	,000076*	,000203*	,000193*	,000930*	,000827*	
hot	8	{8}	,010017*	,026686*	,025172*	,102227	,091957	
hot	9	{9}	,000311*	,000891*	,000845*	,004127*	,003672*	
cold	1	{10}	,835700	,497119	,508962	,176661	,197378	
cold	2	{11}	,299976	,553974	,540183	,847561	,904119	
cold	3	{12}	,520965	,867966	,843916	,546096	,589627	
cold	4	{13}	,286093	,535933	,518402	,879187	,937555	
cold	5	{14}	,218875	,431637	,412390	,954590	,895210	
cold	6	{15}	,211373	,419337	,399769	,936365	,878834	
cold	7	{16}	,203717	,406536	,386778	,919601	,861885	
cold	8	{17}	,009248*	,024736*	,023274*	,096797	,086457	
cold	9	{18}	--	--	--	--	--	

			{6}	{7}	{8}	{9}	{10}	
SMOKING	STIMES			68,25500	62,00000	67,14000	63,53000	74,48000
hot	1	{1}	,026794*	,000076*	,010017*	,000311*	,835700	
hot	2	{2}	,066843	,000203*	,026686*	,000891*	,497119	
hot	3	{3}	,063079	,000193*	,025172*	,000845*	,508962	
hot	4	{4}	,225917	,000930*	,102227	,004127*	,176661	
hot	5	{5}	,205526	,000827*	,091957	,003672*	,197378	
hot	6	{6}		,012583*	,597434	,048667*	,018579*	
hot	7	{7}	,012583*		,033194*	,470132	,000055*	
hot	8	{8}	,597434	,033194*		,115075	,006809*	
hot	9	{9}	,048667*	,470132	,115075		,000210*	
cold	1	{10}	,018579*	,000055*	,006809*	,000210*		
cold	2	{11}	,176106	,000697*	,077727	,003090*	,230093	
cold	3	{12}	,086768	,000275*	,035367*	,001217*	,415991	
cold	4	{13}	,186821	,000740*	,082774	,003277*	,218046	
cold	5	{14}	,236373	,000981*	,107653	,004348*	,163678	
cold	6	{15}	,232278	,000965*	,106214	,004246*	,157743	
cold	7	{16}	,217957	,000930*	,101488	,004069*	,151751	
cold	8	{17}	,584072	,032089*	,958402	,108200	,006280*	
cold	9	{18}	--	--	--	--	--	

			{11}	{12}	{13}	{14}	{15}	
SMOKING	STIMES			71,58500	72,56000	71,48000	71,02000	70,96000
hot	1	{1}	,299976	,520965	,286093	,218875	,211373	
hot	2	{2}	,553974	,867966	,535933	,431637	,419337	
hot	3	{3}	,540183	,843916	,518402	,412390	,399769	
hot	4	{4}	,847561	,546096	,879187	,954590	,936365	
hot	5	{5}	,904119	,589627	,937555	,895210	,878834	
hot	6	{6}	,176106	,086768	,186821	,236373	,232278	
hot	7	{7}	,000697*	,000275*	,000740*	,000981*	,000965*	
hot	8	{8}	,077727	,035367*	,082774	,107653	,106214	
hot	9	{9}	,003090*	,001217*	,003277*	,004348*	,004246*	
cold	1	{10}	,230093	,415991	,218046	,163678	,157743	
cold	2	{11}		,643965	,960299	,811059	,793985	
cold	3	{12}	,643965		,629116	,517641	,504477	
cold	4	{13}	,960299	,629116		,842495	,826839	
cold	5	{14}	,811059	,517641	,842495		,977352	
cold	6	{15}	,793985	,504477	,826839	,977352		
cold	7	{16}	,775082	,490540	,808480	,958072	,977352	
cold	8	{17}	,072413	,032870*	,077419	,103038	,103428	
cold	9	{18}	--	--	--	--	--	

			{16}	{17}	{18}

SMOKING	STIMES		70,90000	67,03000	0,000000
hot	1	{1}	,203717	,009248*	--
hot	2	{2}	,406536	,024736*	--
hot	3	{3}	,386778	,023274*	--
hot	4	{4}	,919601	,096797	--
hot	5	{5}	,861885	,086457	--
hot	6	{6}	,217957	,584072	--
hot	7	{7}	,000930*	,032089*	--
hot	8	{8}	,101488	,958402	--
hot	9	{9}	,004069*	,108200	--
cold	1	{10}	,151751	,006280*	--
cold	2	{11}	,775082	,072413	--
cold	3	{12}	,490540	,032870*	--
cold	4	{13}	,808480	,077419	--
cold	5	{14}	,958072	,103038	--
cold	6	{15}	,977352	,103428	--
cold	7	{16}		,101948	--
cold	8	{17}	,101948		--
cold	9	{18}	--	--	

STAT. GENERAL MANOVA

Duncan test; ASH (new2.sta)
Probabilities for Post Hoc Tests
INTERACTION: 1 x 2

SMOKING	STIMES		{1} 1,716667	{2} 1,605000	{3} 1,915000	{4} 2,035000	{5} 2,245000
hot	1	{1}		,717463	,545282	,363675	,146264
hot	2	{2}	,717463		,361744	,227557	,083681
hot	3	{3}	,545282	,361744		,722283	,350301
hot	4	{4}	,363675	,227557	,722283		,534783
hot	5	{5}	,146264	,083681	,350301	,534783	
hot	6	{6}	,047527*	,025325*	,132677	,227557	,515682
hot	7	{7}	,006281*	,003094*	,020940*	,039599*	,115624
hot	8	{8}	,005480*	,002694*	,018381*	,035220*	,105440
hot	9	{9}	,000521*	,000254*	,001870*	,003734*	,012987*
cold	1	{10}	,458859	,669833	,206189	,122138	,041033*
cold	2	{11}	,286169	,174125	,600969	,845700	,646814
cold	3	{12}	,786994	,552006	,709437	,495598	,214957
cold	4	{13}	,076815	,042068*	,202311	,332684	,691162
cold	5	{14}	,163296	,094145	,383683	,572435	,935394
cold	6	{15}	,471743	,305721	,878678	,820407	,417884
cold	7	{16}	,472575	,306573	,883950	,818679	,415049
cold	8	{17}	,109467	,061325	,274492	,434476	,845700
cold	9	{18}	--	--	--	--	--

SMOKING	STIMES		{6} 2,465000	{7} 2,800000	{8} 2,825000	{9} 3,180000	{10} 1,473333
hot	1	{1}	,047527*	,006281*	,005480*	,000521*	,458859
hot	2	{2}	,025325*	,003094*	,002694*	,000254*	,669833
hot	3	{3}	,132677	,020940*	,018381*	,001870*	,206189
hot	4	{4}	,227557	,039599*	,035220*	,003734*	,122138
hot	5	{5}	,515682	,115624	,105440	,012987*	,041033*
hot	6	{6}		,284201	,276392	,042152*	,011557*
hot	7	{7}	,284201		,935394	,251257	,001315*
hot	8	{8}	,276392	,935394		,257310	,001144*
hot	9	{9}	,042152*	,251257	,257310		,000113*
cold	1	{10}	,011557*	,001315*	,001144*	,000113*	
cold	2	{11}	,292841	,054354	,048658*	,005337*	,090890
cold	3	{12}	,074134	,010465*	,009152*	,000885*	,336978
cold	4	{13}	,770451	,201000	,189641	,026185*	,019729*
cold	5	{14}	,477638	,105440	,095175	,011660*	,046473*
cold	6	{15}	,165951	,026856*	,023762*	,002428*	,170146
cold	7	{16}	,163780	,026831*	,023649*	,002446*	,170579
cold	8	{17}	,625134	,150565	,139216	,017997*	,029444*
cold	9	{18}	--	--	--	--	--

			{11}	{12}	{13}	{14}	{15}

SMOKING	STIMES		2,095000	1,800000	2,375000	2,220000	1,965000
hot	1	{1}	,286169	,786994	,076815	,163296	,471743
hot	2	{2}	,174125	,552006	,042068*	,094145	,305721
hot	3	{3}	,600969	,709437	,202311	,383683	,878678
hot	4	{4}	,845700	,495598	,332684	,572435	,820407
hot	5	{5}	,646814	,214957	,691162	,935394	,417884
hot	6	{6}	,292841	,074134	,770451	,477638	,165951
hot	7	{7}	,054354	,010465*	,201000	,105440	,026856*
hot	8	{8}	,048658*	,009152*	,189641	,095175	,023762*
hot	9	{9}	,005337*	,000885*	,026185*	,011660*	,002428*
cold	1	{10}	,090890	,336978	,019729*	,046473*	,170146
cold	2	{11}		,399170	,417884	,685569	,691162
cold	3	{12}	,399170		,117118	,238123	,625271
cold	4	{13}	,417884	,117118		,646307	,249068
cold	5	{14}	,685569	,238123	,646307		,451848
cold	6	{15}	,691162	,625271	,249068	,451848	
cold	7	{16}	,689241	,625134	,246061	,451450	,987152
cold	8	{17}	,534783	,163780	,820407	,795010	,332684
cold	9	{18}	--	--	--	--	--

SMOKING	STIMES		{16} 1,960000	{17} 2,305000	{18} 0,000000
hot	1	{1}	,472575	,109467	--
hot	2	{2}	,306573	,061325	--
hot	3	{3}	,883950	,274492	--
hot	4	{4}	,818679	,434476	--
hot	5	{5}	,415049	,845700	--
hot	6	{6}	,163780	,625134	--
hot	7	{7}	,026831*	,150565	--
hot	8	{8}	,023649*	,139216	--
hot	9	{9}	,002446*	,017997*	--
cold	1	{10}	,170579	,029444*	--
cold	2	{11}	,689241	,534783	--
cold	3	{12}	,625134	,163780	--
cold	4	{13}	,246061	,820407	--
cold	5	{14}	,451450	,795010	--
cold	6	{15}	,987152	,332684	--
cold	7	{16}		,329418	--
cold	8	{17}	,329418		--
cold	9	{18}	--	--	

STAT. Duncan test; PROTEIN (new2.sta)
GENERAL Probabilities for Post Hoc Tests
MANOVA INTERACTION: 1 x 2

SMOKING	STIMES		{1}	{2}	{3}	{4}	{5}	
				22,01667	20,29500	22,13500	23,34500	23,40500
hot	1	{1}		,607044	,969189	,700708	,690264	
hot	2	{2}	,607044		,589464	,385828	,377249	
hot	3	{3}	,969189	,589464		,722823	,713194	
hot	4	{4}	,700708	,385828	,722823		,984411	
hot	5	{5}	,690264	,377249	,713194	,984411		
hot	6	{6}	,064456	,025013*	,067612	,116120	,113052	
hot	7	{7}	,056607	,021730*	,059562	,105184	,104053	
hot	8	{8}	,012864*	,004532*	,013498*	,024997*	,024495*	
hot	9	{9}	,115393	,047207*	,120815	,197706	,190374	
cold	1	{10}	,710860	,856009	,699402	,479358	,470637	
cold	2	{11}	,445223	,770837	,430378	,267802	,261263	
cold	3	{12}	,288837	,543194	,277900	,163979	,159796	
cold	4	{13}	,909468	,544246	,936445	,759620	,758813	
cold	5	{14}	,706246	,869952	,688652	,465746	,456333	
cold	6	{15}	,948046	,574745	,975280	,738914	,730607	
cold	7	{16}	,923011	,554135	,947641	,758813	,752470	
cold	8	{17}	,480816	,241011	,500111	,726191	,725080	
cold	9	{18}	--	--	--	--	--	

SMOKING	STIMES		{6}	{7}	{8}	{9}	{10}	
				28,83500	29,09500	31,60000	27,72000	20,88333
hot	1	{1}	,064456	,056607	,012864*	,115393	,710860	
hot	2	{2}	,025013*	,021730*	,004532*	,047207*	,856009	
hot	3	{3}	,067612	,059562	,013498*	,120815	,699402	
hot	4	{4}	,116120	,105184	,024997*	,197706	,479358	
hot	5	{5}	,113052	,104053	,024495*	,190374	,470637	
hot	6	{6}		,932181	,396360	,715315	,034265*	
hot	7	{7}	,932181		,415848	,671480	,029917*	
hot	8	{8}	,396360	,415848		,251585	,006342*	
hot	9	{9}	,715315	,671480	,251585		,063803	
cold	1	{10}	,034265*	,029917*	,006342*	,063803		
cold	2	{11}	,014782*	,012795*	,002568*	,028592*	,658556	
cold	3	{12}	,007585*	,006544*	,001268*	,015060*	,453575	
cold	4	{13}	,072126	,064635	,014389*	,127972	,657839	
cold	5	{14}	,033107*	,028846*	,006144*	,061639	,977014	
cold	6	{15}	,069701	,061643	,013898*	,124375	,687190	
cold	7	{16}	,072432	,064410	,014468*	,128840	,666944	
cold	8	{17}	,186433	,175032	,044987*	,295417	,309112	
cold	9	{18}	--	--	--	--	--	

SMOKING	STIMES		{11}	{12}	{13}	{14}	{15}	
				19,40500	18,32000	22,41000	20,79500	22,23000
hot	1	{1}	,445223	,288837	,909468	,706246	,948046	
hot	2	{2}	,770837	,543194	,544246	,869952	,574745	
hot	3	{3}	,430378	,277900	,936445	,688652	,975280	
hot	4	{4}	,267802	,163979	,759620	,465746	,738914	
hot	5	{5}	,261263	,159796	,758813	,456333	,730607	
hot	6	{6}	,014782*	,007585*	,072126	,033107*	,069701	
hot	7	{7}	,012795*	,006544*	,064635	,028846*	,061643	
hot	8	{8}	,002568*	,001268*	,014389*	,006144*	,013898*	
hot	9	{9}	,028592*	,015060*	,127972	,061639	,124375	
cold	1	{10}	,658556	,453575	,657839	,977014	,687190	
cold	2	{11}		,722636	,392668	,668122	,418034	
cold	3	{12}	,722636		,250857	,460939	,268962	
cold	4	{13}	,392668	,250857		,641988	,956646	
cold	5	{14}	,668122	,460939	,641988		,673700	
cold	6	{15}	,418034	,268962	,956646	,673700		
cold	7	{16}	,400928	,256660	,984411	,651941	,968755	
cold	8	{17}	,160559	,094170	,536785	,298539	,514673	
cold	9	{18}	--	--	--	--	--	

			{16}	{17}	{18}

SMOKING		STIMES		22,35000	24,48000	0,000000
hot	1	{1}	,923011	,480816	--	
hot	2	{2}	,554135	,241011	--	
hot	3	{3}	,947641	,500111	--	
hot	4	{4}	,758813	,726191	--	
hot	5	{5}	,752470	,725080	--	
hot	6	{6}	,072432	,186433	--	
hot	7	{7}	,064410	,175032	--	
hot	8	{8}	,014468*	,044987*	--	
hot	9	{9}	,128840	,295417	--	
cold	1	{10}	,666944	,309112	--	
cold	2	{11}	,400928	,160559	--	
cold	3	{12}	,256660	,094170	--	
cold	4	{13}	,984411	,536785	--	
cold	5	{14}	,651941	,298539	--	
cold	6	{15}	,968755	,514673	--	
cold	7	{16}		,532594	--	
cold	8	{17}	,532594		--	
cold	9	{18}	--	--		

STAT. GENERAL MANOVA

Duncan test; FAT (new2.sta)
Probabilities for Post Hoc Tests
INTERACTION: 1 x 2

SMOKING		STIMES	{1}	{2} 3,746667	{3} 4,525000	{4} 6,465000	{5} 6,630000	6,600000
hot	1	{1}		,539991	,062362	,050121	,052009	
hot	2	{2}	,539991		,169356	,138751	,143891	
hot	3	{3}	,062362	,169356		,896643	,909995	
hot	4	{4}	,050121	,138751	,896643		,980032	
hot	5	{5}	,052009	,143891	,909995	,980032		
hot	6	{6}	,030794*	,089660	,696531	,772723	,767239	
hot	7	{7}	,136109	,331441	,628460	,545309	,560499	
hot	8	{8}	,221239	,491287	,437623	,371374	,383163	
hot	9	{9}	,070409	,190337	,932225	,836713	,853764	
cold	1	{10}	,917756	,584467	,073385	,059184	,061369	
cold	2	{11}	,319252	,654700	,310300	,259120	,268139	
cold	3	{12}	,083614	,221015	,853764	,760290	,777040	
cold	4	{13}	,086073	,226508	,833847	,740689	,757516	
cold	5	{14}	,089317	,233186	,808234	,715479	,732413	
cold	6	{15}	,068174	,184186	,953678	,859188	,873444	
cold	7	{16}	,068897	,186394	,946780	,850882	,867893	
cold	8	{17}	,067769	,182681	,956643	,861046	,874692	
cold	9	{18}	--	--	--	--	--	

SMOKING		STIMES	{6} 6,975000	{7} 5,805000	{8} 5,400000	{9} 6,350000	{10} 3,870000
hot	1	{1}	,030794*	,136109	,221239	,070409	,917756
hot	2	{2}	,089660	,331441	,491287	,190337	,584467
hot	3	{3}	,696531	,628460	,437623	,932225	,073385
hot	4	{4}	,772723	,545309	,371374	,836713	,059184
hot	5	{5}	,767239	,560499	,383163	,853764	,061369
hot	6	{6}		,394352	,257701	,646815	,036580*
hot	7	{7}	,394352		,734668	,682464	,154650
hot	8	{8}	,257701	,734668		,482005	,247629
hot	9	{9}	,646815	,682464	,482005		,082275
cold	1	{10}	,036580*	,154650	,247629	,082275	
cold	2	{11}	,174503	,557352	,775921	,345923	,351241
cold	3	{12}	,578110	,753895	,541278	,909995	,097231
cold	4	{13}	,560499	,767239	,551505	,893531	,099634
cold	5	{14}	,538178	,782330	,562617	,870458	,102728
cold	6	{15}	,664179	,666754	,468795	,976400	,079993
cold	7	{16}	,658809	,672236	,473723	,983386	,080706
cold	8	{17}	,666078	,662542	,465177	,970425	,079606
cold	9	{18}	--	--	--	--	--

			{11}	{12}	{13}	{14}	{15}

SMOKING	STIMES		5,060000	6,215000	6,180000	6,135000	6,390000
hot	1	{1}	,319252	,083614	,086073	,089317	,068174
hot	2	{2}	,654700	,221015	,226508	,233186	,184186
hot	3	{3}	,310300	,853764	,833847	,808234	,953678
hot	4	{4}	,259120	,760290	,740689	,715479	,859188
hot	5	{5}	,268139	,777040	,757516	,732413	,873444
hot	6	{6}	,174503	,578110	,560499	,538178	,664179
hot	7	{7}	,557352	,753895	,767239	,782330	,666754
hot	8	{8}	,775921	,541278	,551505	,562617	,468795
hot	9	{9}	,345923	,909995	,893531	,870458	,976400
cold	1	{10}	,351241	,097231	,099634	,102728	,079993
cold	2	{11}		,394129	,402992	,413329	,335088
cold	3	{12}	,394129		,976705	,950310	,895079
cold	4	{13}	,402992	,976705		,970032	,876618
cold	5	{14}	,413329	,950310	,970032		,850882
cold	6	{15}	,335088	,895079	,876618	,850882	
cold	7	{16}	,339120	,899757	,882850	,859188	,990070
cold	8	{17}	,332148	,891199	,871144	,845296	,993412
cold	9	{18}	--	--	--	--	--

			{16}	{17}	{18}
SMOKING	STIMES		6,375000	6,400000	0,000000
hot	1	{1}	,068897	,067769	--
hot	2	{2}	,186394	,182681	--
hot	3	{3}	,946780	,956643	--
hot	4	{4}	,850882	,861046	--
hot	5	{5}	,867893	,874692	--
hot	6	{6}	,658809	,666078	--
hot	7	{7}	,672236	,662542	--
hot	8	{8}	,473723	,465177	--
hot	9	{9}	,983386	,970425	--
cold	1	{10}	,080706	,079606	--
cold	2	{11}	,339120	,332148	--
cold	3	{12}	,899757	,891199	--
cold	4	{13}	,882850	,871144	--
cold	5	{14}	,859188	,845296	--
cold	6	{15}	,990070	,993412	--
cold	7	{16}		,985248	--
cold	8	{17}	,985248		--
cold	9	{18}	--	--	

STAT. GENERAL MANOVA

Duncan test; SALT (new2.sta)
Probabilities for Post Hoc Tests
INTERACTION: 1 x 2

			{1}	{2}	{3}	{4}	{5}
SMOKING	STIMES			,5150000	,5700000	,5800000	,9000000	1,195000
hot	1	{1}		,696559	,663815	,018392*	,000312*
hot	2	{2}	,696559		,943432	,035237*	,000654*
hot	3	{3}	,663815	,943432		,032764*	,000690*
hot	4	{4}	,018392*	,035237*	,032764*		,069582
hot	5	{5}	,000312*	,000654*	,000690*	,069582	
hot	6	{6}	,000140*	,000288*	,000312*	,033122*	,663815
hot	7	{7}	,000067*	,000137*	,000151*	,015824*	,419071
hot	8	{8}	,000028*	,000050*	,000054*	,005275*	,200942
hot	9	{9}	,000011*	,000011*	,000011*	,000013*	,000037*
cold	1	{10}	,731718	,490844	,464127	,010093*	,000166*
cold	2	{11}	,002901*	,005926*	,005782*	,361035	,289496
cold	3	{12}	,000593*	,001231*	,001249*	,117272	,712930
cold	4	{13}	,000313*	,000639*	,000666*	,068836	,971774
cold	5	{14}	,000232*	,000485*	,000522*	,053433	,859138
cold	6	{15}	,000067*	,000139*	,000150*	,015873*	,428475
cold	7	{16}	,000079*	,000150*	,000162*	,017490*	,457659
cold	8	{17}	,000070*	,000140*	,000159*	,016448*	,433676
cold	9	{18}	--	--	--	--	--

			{6}	{7}	{8}	{9}	{10}

SMOKING	STIMES		1,260000	1,325000	1,405000	2,064500	,4666667
hot	1	{1}	,000140*	,000067*	,000028*	,000011*	,731718
hot	2	{2}	,000288*	,000137*	,000050*	,000011*	,490844
hot	3	{3}	,000312*	,000151*	,000054*	,000011*	,464127
hot	4	{4}	,033122*	,015824*	,005275*	,000013*	,010093*
hot	5	{5}	,663815	,419071	,200942	,000037*	,000166*
hot	6	{6}		,679192	,365109	,000062*	,000080*
hot	7	{7}	,679192		,571386	,000118*	,000039*
hot	8	{8}	,365109	,571386		,000282*	,000019*
hot	9	{9}	,000062*	,000118*	,000282*		,000011*
cold	1	{10}	,000080*	,000039*	,000019*	,000011*	
cold	2	{11}	,156967	,081199	,030814*	,000016*	,001530*
cold	3	{12}	,446926	,258801	,114195	,000028*	,000320*
cold	4	{13}	,650102	,403752	,192673	,000036*	,000167*
cold	5	{14}	,776523	,509787	,254775	,000044*	,000125*
cold	6	{15}	,688195	,974995	,582095	,000107*	,000040*
cold	7	{16}	,722863	,924876	,546121	,000099*	,000043*
cold	8	{17}	,697326	,971774	,570294	,000118*	,000041*
cold	9	{18}	--	--	--	--	--

			{11}	{12}	{13}	{14}	{15}	
SMOKING	STIMES		1,030000	1,140000	1,190000	1,220000	1,320000	
hot	1	{1}	,002901*	,000593*	,000313*	,000232*	,000067*	
hot	2	{2}	,005926*	,001231*	,000639*	,000485*	,000139*	
hot	3	{3}	,005782*	,001249*	,000666*	,000522*	,000150*	
hot	4	{4}	,361035	,117272	,068836	,053433	,015873*	
hot	5	{5}	,289496	,712930	,971774	,859138	,428475	
hot	6	{6}	,156967	,446926	,650102	,776523	,688195	
hot	7	{7}	,081199	,258801	,403752	,509787	,974995	
hot	8	{8}	,030814*	,114195	,192673	,254775	,582095	
hot	9	{9}	,000016*	,000028*	,000036*	,000044*	,000107*	
cold	1	{10}	,001530*	,000320*	,000167*	,000125*	,000040*	
cold	2	{11}		,438160	,289375	,233036	,083139	
cold	3	{12}	,438160		,722863	,604380	,267378	
cold	4	{13}	,289375	,722863		,840936	,415803	
cold	5	{14}	,233036	,604380	,840936		,517822	
cold	6	{15}	,083139	,267378	,415803	,517822		
cold	7	{16}	,091232	,290212	,446926	,548001	,943432	
cold	8	{17}	,084794	,269828	,419071	,525334	1,000000	
cold	9	{18}	--	--	--	--	--	

			{16}	{17}	{18}	
SMOKING	STIMES		1,310000	1,320000	0,000000	
hot	1	{1}	,000079*	,000070*	--	
hot	2	{2}	,000150*	,000140*	--	
hot	3	{3}	,000162*	,000159*	--	
hot	4	{4}	,017490*	,016448*	--	
hot	5	{5}	,457659	,433676	--	
hot	6	{6}	,722863	,697326	--	
hot	7	{7}	,924876	,971774	--	
hot	8	{8}	,546121	,570294	--	
hot	9	{9}	,000099*	,000118*	--	
cold	1	{10}	,000043*	,000041*	--	
cold	2	{11}	,091232	,084794	--	
cold	3	{12}	,290212	,269828	--	
cold	4	{13}	,446926	,419071	--	
cold	5	{14}	,548001	,525334	--	
cold	6	{15}	,943432	1,000000	--	
cold	7	{16}		,947200	--	
cold	8	{17}	,947200		--	
cold	9	{18}	--	--		

STAT. **Duncan test; TPC (new.sta)**

GENERAL			**Probabilities for Post Hoc Tests**				
MANOVA			INTERACTION: 1 x 2				
			{1}	{2}	{3}	{4}	{5}
SMOKING		STIME	0,000000	0,000000	10,00000	275,0000	0,000000
hot	1	{1}		1,000000	1,000000	1,000000	1,000000
hot	2	{2}	1,000000		1,000000	1,000000	1,000000
hot	3	{3}	1,000000	1,000000		1,000000	1,000000
hot	4	{4}	1,000000	1,000000	1,000000		1,000000

hotv	1	{5}		1,000000	1,000000	1,000000	1,000000
hotv	2	{6}	1,000000	1,000000	1,000000	1,000000	1,000000
hotv	3	{7}	1,000000	1,000000	,999999	1,000000	1,000000
hotv	4	{8}	1,000000	1,000000	,999996	1,000000	1,000000
cold	1	{9}	1,000000	1,000000	1,000000	,999992	1,000000
cold	2	{10}	1,000000	1,000000	1,000000	,996787	1,000000
cold	3	{11}	,264981	,265394	,260205	,234808	,265315
SMOKING		STIME	0,000000	0,000000	10,00000	275,0000	0,000000
cold	4	{12}	,004046*	,003918*	,003212*	,002362*	,003773*
			{6}	{7}	{8}	{9}	{10}
SMOKING		STIME	0,000000	0,000000	50,00000	200,0000	25525,00
hot	1	{1}	1,000000	1,000000	1,000000	1,000000	1,000000
hot	2	{2}	1,000000	1,000000	1,000000	1,000000	1,000000
hot	3	{3}	1,000000	,999999	,999996	1,000000	1,000000
hot	4	{4}	1,000000	1,000000	1,000000	,999992	,996787
hotv	1	{5}	1,000000	1,000000	1,000000	1,000000	1,000000
hotv	2	{6}		1,000000	1,000000	1,000000	1,000000
hotv	3	{7}	1,000000		1,000000	1,000000	1,000000
hotv	4	{8}	1,000000	1,000000		,999984	1,000000
cold	1	{9}	1,000000	1,000000	,999984		,997050
cold	2	{10}		1,000000	1,000000	1,000000	,997050
cold	3	{11}	,264624	,263049	,255466	,247719	,213274
cold	4	{12}	,003609*	,003424*	,002966*	,002686*	,001989*
				{11}	{12}		
SMOKING		STIME		7810000,	2334E4		
hot	1	{1}		,264981	,004046*		
hot	2	{2}		,265394	,003918*		
hot	3	{3}		,260205	,003212*		
hot	4	{4}		,234808	,002362*		
hotv	1	{5}		,265315	,003773*		
hotv	2	{6}		,264624	,003609*		
hotv	3	{7}		,263049	,003424*		
hotv	4	{8}		,255466	,002966*		
cold	1	{9}		,247719	,002686*		
cold	2	{10}		,213274	,001989*		
cold	3	{11}			,020488*		
cold	4	{12}		,020488*			
STAT.				**Duncan test; LAB (new.sta)**			
GENERAL				**Probabilities for Post Hoc Tests**			
MANOVA				INTERACTION: 1 x 2			
			{1}	{2}	{3}	{4}	{5}
SMOKING		STIME	0,000000	0,000000	0,000000	300,0000	50,00000
hot	1	{1}		1,000000	1,000000	1,000000	1,000000
hot	2	{2}	1,000000		1,000000	1,000000	1,000000
hot	3	{3}	1,000000	1,000000		1,000000	1,000000
hot	4	{4}	1,000000	1,000000	1,000000		1,000000
hotv	1	{5}		1,000000	1,000000	1,000000	1,000000
hotv	2	{6}	1,000000	1,000000	1,000000	1,000000	1,000000
SMOKING		STIME	0,000000	0,000000	0,000000	300,0000	50,00000
hotv	3	{7}	1,000000	1,000000	1,000000	1,000000	,999999
hotv	4	{8}	1,000000	1,000000	1,000000	1,000000	1,000000
cold	1	{9}	1,000000	1,000000	1,000000	,999992	1,000000
cold	2	{10}	1,000000	1,000000	1,000000	,999490	1,000000
cold	3	{11}	,381033	,381920	,382424	,353556	,378795
cold	4	{12}	,018908*	,018555*	,018128*	,012715*	,016236*
			{6}	{7}	{8}	{9}	{10}
SMOKING		STIME	0,000000	10,00000	50,00000	50,00000	16000,00
hot	1	{1}	1,000000	1,000000	1,000000	1,000000	1,000000
hot	2	{2}	1,000000	1,000000	1,000000	1,000000	1,000000
hot	3	{3}	1,000000	1,000000	1,000000	1,000000	1,000000
hot	4	{4}	1,000000	1,000000	1,000000	,999992	,999490
hotv	1	{5}	1,000000	,999999	1,000000	1,000000	1,000000
hotv	2	{6}		1,000000	1,000000	1,000000	1,000000
hotv	3	{7}	1,000000		1,000000	1,000000	1,000000
hotv	4	{8}	1,000000	1,000000		1,000000	1,000000
cold	1	{9}	1,000000	1,000000	1,000000		,999454
cold	2	{10}		1,000000	1,000000	1,000000	,999454
cold	3	{11}	,382244	,381172	,374442	,366858	,328982
cold	4	{12}	,017614*	,016993*	,015304*	,014155*	,010895*
				{11}	{12}		

SMOKING	STIME	2041E4	6129E4
hot	1 {1}	,381033	,018908*
hot	2 {2}	,381920	,018555*
hot	3 {3}	,382424	,018128*
hot	4 {4}	,353556	,012715*
hotv	1 {5}	,378795	,016236*
hotv	2 {6}	,382244	,017614*
hotv	3 {7}	,381172	,016993*
hotv	4 {8}	,374442	,015304*
cold	1 {9}	,366858	,014155*
cold	2 {10}	,328982	,010895*
cold	3 {11}		,061388
cold	4 {12}	,061388	

Table 18: Result of t-Test for TVB (cold smoked fish and hot smoked with packing)

t-Test: Two-Sample Assuming Unequal Variances
t- Test TVB cold + hot with packing

	Variable 1	*Variable 2*
Mean	27,31889	30,36556
Variance	30,76939	94,32213
Observations	9	9
Hypothesized Mean Difference	0	
df	13	
t Stat	-0,81721	
P(T<=t) one-tail	0,214267	
t Critical one-tail	1,770932	
P(T<=t) two-tail	0,428533	
t Critical two-tail	2,160368	
Correlation	0,533673	

Table 19: Result of t-Test for TVB (cold smoked fish and hot smoked without packing)

t-Test: Two-Sample Assuming Unequal Variances
t-Test TVB cold + hot without vacuum

	Variable 1	*Variable 2*
Mean	29,90778	30,36556
Variance	80,97962	94,32213
Observations	9	9
Hypothesized Mean Difference	0	
df	16	
t Stat	-0,10372	
P(T<=t) one-tail	0,459338	
t Critical one-tail	1,745884	
P(T<=t) two-tail	0,918677	
t Critical two-tail	2,119905	

Table 20: Result of t-Test for TVB (hot smoked fish with and without packing)

t-Test: Two-Sample Assuming Unequal Variances

t-Test TVB hot with and without vacuum

	Variable 1	*Variable 2*
Mean	27,31889	29,90778
Variance	30,76939	80,97962
Observations	9	9
Hypothesized Mean Difference	0	
df	13	
t Stat	-0,7347	
P(T<=t) one-tail	0,237782	
t Critical one-tail	1,770932	
P(T<=t) two-tail	0,475564	
t Critical two-tail	2,160368	
Correlation	0,826338	

Table 21: Result of t-Test for Salt for hot and cold smoking

t-Test: Two-Sample Assuming Unequal Variances

t-Test: Salt

	Variable 1	*Variable 2*
Mean	1,060211	1,085882
Variance	0,259824	0,102526
Observations	19	17
Hypothesized Mean Difference	0	
df	31	
t Stat	-0,18288	
P(T<=t) one-tail	0,428043	
t Critical one-tail	1,695519	
P(T<=t) two-tail	0,856085	
t Critical two-tail	2,039515	
correlation	0,690	

Table 22: Result of t-Test for fat for hot and cold smoking

t-Test: Two-Sample Assuming Unequal Variances

t-Test: fat

	Variable 1	*Variable 2*
Mean	5,723158	5,712941
Variance	1,370423	2,551022
Observations	19	17
Hypothesized Mean Difference	0	
df	29	
t Stat	0,021675	
P(T<=t) one-tail	0,491428	
t Critical one-tail	1,699127	
P(T<=t) two-tail	0,982856	
t Critical two-tail	2,045231	
Correlation	0,478	

Table 23: Result of t-Test for protein for hot and cold smoking

t-Test: Two-Sample Assuming Unequal Variances

t-Test: protein

	Variable 1	*Variable 2*
Mean	25,20579	21,33118
Variance	16,80121	11,96185
Observations	19	17
Hypothesized Mean Difference	0	
df	34	
t Stat	3,074787	
P(T<=t) one-tail	0,002069	
t Critical one-tail	1,690923	
P(T<=t) two-tail	0,004137	
t Critical two-tail	2,032243	
correlation	0,555	

Table 24: Result of t-Test for ash for hot and cold smoking

t-Test: Two-Sample Assuming Unequal Variances

t-Test: Ash

	Variable 1	*Variable 2*
Mean	2,28	1,991765
Variance	0,367056	0,112228
Observations	19	17
Hypothesized Mean Difference	0	
df	29	
t Stat	1,790304	
P(T<=t) one-tail	0,041926	
t Critical one-tail	1,699127	
P(T<=t) two-tail	0,083852	
t Critical two-tail	2,045231	
Correlation	0,260	

Table 25: Result of t-Test for water for hot and cold smoking

t-Test: Two-Sample Assuming Unequal Variances

t-Test: water

	Variable 1	*Variable 2*
Mean	69,51105	71,44176
Variance	22,27938	4,648465
Observations	19	17
Hypothesized Mean Difference	0	
df	26	
t Stat	-1,60556	
P(T<=t) one-tail	0,060224	
t Critical one-tail	1,705616	
P(T<=t) two-tail	0,120449	
t Critical two-tail	2,055531	
correlation	0,561	

T-Test for Total count hot smoked product with and without packing

STAT. **T-test for Independent Samples (new8.sta)**
BASIC **Note: Variables were treated as independent samples**
STATS

Group 1	vs. Group 2	Mean Group 1	Mean Group 2	t-value	df	p
VAR2	vs. VAR6	30,00000	11,11111	1,041359	16	,313190

t-Test forTotal count Hot smoked product without packing and cold smoked product

Variable	Mean	Std.Dv.	N	Diff.	Diff.	t	df
VAR2	30,	43,					
VAR4	350591E2	525104E2	9	-35060E3	525104E2	-2,00298	8

T-Test for Total count hot smoked product with packing and cold smoked product

Group 1	vs. Group 2	Mean Group 1	Mean Group 2	t-value	df	p
VAR4	vs. VAR6	350591E2	11,11111	2,002980	16	,062422

T-Test for lactic acid bacteria Hot smoked product without packing and cold smoked product

Group 1	vs. Group 2	Mean Group 1	Mean Group 2	t-value	df	p
TPC_COLD	vs. TPC_HOTV	132080E2	11,11111	1,743418	16	,100441

T-Test for lactic acid bacteria Hot smoked product with packing and cold smoked product

Mean Group 1	vs. Group 2	Mean Group 1	Group 2	t-value	df	p
TPC_COLD	vs. TPC_HOT	132080E2	63,33333	1,743411	16	,100442