

PACTORS CAUSING CLOUDINESS IN **PICKLE BRINES**

Thesis for the Degree of M. S. MICHIGAN STATE COLLEGE Marvin Nelson Kragt 19B1

THESIS

This is to certify that the

thesis entitled Factors Causing Cloudiness in Pickle Brines.

presented by

Marvin N. Kragt

has been accepted towards fulfillment of the requirements for

degree in Bacty. & P.H. $M_{\bullet} S_{\bullet}$

Fabian

Major professor

Date May 14, 1951

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FACTORS CAUSING CLOUDINESS IN PICKLE BRINES

By

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A.IEESIS

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IN TRODUCTION

The cloudiness in pickle brines is an important problem in the pickle industry. It is not only a problem of the processor but it has also become a problem to the consumer. Today with the shortage of tin, more and more pickles are being packed in glass. which allows the consumer to note any cloudiness in the brine. The general public has been educated to the fact that cloudiness in any food product usually indicates spoilage. Pickles that contain sediment or that are turbid usually influences a customer to buy the pickles that have the clear brine. An examination of pickle products on the grocer shelves show that practically all fresh pasteurized or processed dill pickles show cloudiness or sediment ranging from slight to a heavy sediment. While other kinds of pickles have been observed in which this condition was noticable. it is not as prevalent as in the dill pickles.

Since a clear brine is essential for consumer acceptance of pickle products, a study was made of the bacterial, chemical and physical factors causing turbidity in pickles. This study was confined mostly to fresh pasteurized and processed dill pickles. because the most sediment is found in them.

LITERATURE REVIET

Etchells and Gorcsline (1940) observed turbidity in opened jars of fresh cucumber dill pickles. Bacterial counts proved negative and results indicated that the turbidity was due to soaking the slices in brine of 30° salometer for different lengths of time. Slices that had been previously soaked the shortest time had the greatest turbidity in the jars, indicating that there had been less material removed from the pickles before packing them into the jar. 'When this precipitate was tested with Millions Reagent for protein, results were positive indicating a protein precipitate.

Krants (1928) studying vegetable gums and mineral oil emulsions noted that the range of greatest stability of either vegetable or mineral oil emulsions prepared with acacia was obtained when the pH of the outer phase was between pH 2 and pH 10. With gum tragacanth the range was from pH 1.9 and 2.3. The stable range of acacia emulsions was between $pH 4.4$ and 4.28 , and with tragacanth the stable point was approximately pH 2.5.

Bennett (1947) showed that the addition of electrolytes may act upon an emulsifying agent. If this happens, the electrolyte induces coagulation (salting out) or a change of the chemical nature of the emulsifying agent, thereby forming a precipitate. However, it is also possible that small quantities of electrolytes may stabilise emulsions. The presence of salts, especially calcium, magnesium, aluminum, copper, iron, sine, bismuth, mercury, are likely to destroy emulsions.

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Cobb (1946) listed the factors which break down emulsions as:

- 1. Addition of electrolytes such as inorganic acids and salts. Adding electrolytes, after the emulsion was completed, was in many cases, less apt to result in breaking the emulsion. It must be remembered that the of there is no such a thing as, precipitation, a little bit of a colloid system - it is all or nothing.
- 2. Hardness of water the softer the water the better, with distilled water representing the optimum.
- 3. Oxidation of even fairly inert material in finely divided form is rapid and destructive. Agitation should be carried out in a way to prevent inclusion of large quantities of air in the emulsion.

Weyl (1877) stated that the chief protein found in mustard seed appears to be a globulin protein.

Hones (1949) found vegetable guns to be composed of carbon, hydrogen and oxygen with small quantities of mineral matter and sometimes a little nitrogen. On hydrolysis with dilute mineral acids the gums form various sugars such as pentoses, arabinose, sylose, tragacanthose, and the hexose, galactose. Not all the gum is converted into sugars but usually about 20 per cent resists treatnent.

Icodnan (1941) gives the general composition of mustard seed as containing considerable quantities of non-volatile oil, and protein material. This non-volatile oil is entirely lacking in

pungency, being a tasteless oil somewhat like olive oil. The active constituent of mustard is the volatile oil which is not present in the mustard as such but is developed by an enzyme in an aqueous solution. The actual substance present is singrin or potassium myronate (KC₁₀ H₁₆ HS₂ O₉), a glucoside which by hydrolysis, through the agency of the enzymes also present, splits into glucose, potassium acid sulphate, and allyl isothiocyanate $(C_3 H_5 CNS)$. The latter is the pungent volatile mustard oil obtained from black mustard seeds.

KXPERIMENTAL PROCEDURE

1. Bacteriological studies

Quart Jars of pasteurized dills were received which had a cloudy brine. These were Just a few of the many other jars of processed and pasteurized dill pickles, that had been cloudy when received at the laboratory for analysis. It was decided that the experimental work should first include a microbiological examination of all Jars.

The various cloudy brines from the Jars were plated on three media to determine if the sediment was of bacterial origin. Tryptone glucose yeast extract agar was used to determine peptonizers, acid bacteria, and inert bacteria. The composition of the

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 q agar
(T.G.Y.E.) used was as follows:

Tryptone............ 10 grams Yeast extract........ 5 grams Beef extract........ 3 grams K_2 HPO₄................ 1 gram Glucose............. 1 gram Agar................ 16 grams Distilled water.1000 ml .

Brcm-cresol-purple. . 2 ml. of 1.6% solution One ml. of sterile skim milk and one m1. of sterile pickle brine were added as the plates were poured. It was noted that the sterile brine seemed to increase colony size of organisms.

Potato dextrose agar (P.D.A.) was used to determine the presence of yeasts. Its composition was as follows:

> Infusion from potato... 200 grams Dextrose............... 20 grams Agar................... 15 grams Distilled water.1000 ml.

The medium was acidified to a pH of 3. 5 with tartaric acid. lbmato juice agar (T.J.A.) was used to determine the lactic

acid bacteria. Its composition was as follows: Tomato juice (400 ml). . 20 grams Peptone................ 10 grams Peptonised milk. 10 grams Agar................... 16 grams Distilled water........1000 ml.

The T.G.Y.E. and T.J.A. plates were incubated for 48 hours at 35°C., and the P.D.A. plates were incubated at room temperature for five days.

A yeast extract was used as basal medium for the sugar reactions of the bacteria. Its composition was as follows:

In preparing the carbohydrate for the fermentation studies, the tubes with inserts were sterilised, the basic medium autoclaved, and then the carbon compounds were added in one percent concentration. Seven ml. was then pipetted into each tube and this was again autoclaved at 10 pounds pressure for 10 minutes.

Bergey's Manual of Determinative Bacteriology, sixth edition was used to identify the organisms. (9) .

The percent salt was determined by titrating with standard silver nitrate solution using dichlorofluorescein indicator.

Acidity, calculated as grains acetic acid was determined by using a 0.1666 N. NaOH solution and phenclphthalein indicator. The pH was determined by a Beckman pH meter.

11. Physical studies

Processed dill and fresh pasteurized dill brines were used in these experiments and standardised as shall be seen presently.

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The dispersing agents used for the essential oils consisted of a variety of vegetable gums, tweens and salt in the form of soluble oil of spice, 8.0.8. These agents containing the spices were that added to the two pickle brines in the ratio of 133000. The different combinations of brines and spices were placed in jars, sealed and pasteurized at 165° F. for 15 minutes.

After one week the jars containing spices and brines were thoroughly mixed by shaking 25 times and examined for cloudiness by means of the photolometer.

The cloudiness was measured by means of a Cenco-Sheard Sanford photolometer Model No. 12335. The photolometer was standardized to 100 with distilled water, and the brine readings were determined on comparison with distilled water. Tubular cells and adaptors were used with the photelometer and a lantern red filter no. 2408.

III. Chemical studies

In order to standardize all the brines, the ingredients were added in a constant amount.

- 1. Vinegar to give a dilution of 6.6 grain acid for fresh pasteurized dill brine, and eight grain acid for processed dill brine.
- 2. Salt to give a 2.5 per cent dilution for fresh pasteurized dill brine and four per cent dilution for processed dill brine.
- 3. Alum 0.2 per cent Al_2 (SO_4)₃ 18H₂O, so that the final result would be 0.1 per cent Δl_2 (304) $_5$.
- 4. Spices whole and powdered spices were added to give one ml. to each pint Jar.
- 5. Spice oils were added to give a 1: 3000 dilution of spice oils in the brines.
- 8. Vegetable guns in oil emulsions
	- a. Mixed gun *
		- 0.1 part vegetable gum
		- 1 part oil (dill, pepper, cloves)
		- 2 parts water

Resulting ratio of gun in brine \bullet 1: 12,000

- b. Gum arabic
	- 1 part vegetable gum
	- 4 parts oil
	- 8 parts water

Resulting ratio of gum in brine \bullet 1: 12,000

- c. Gun tragacanth
	- 3.7 parts gum
	- 12.5 parts oil
	- 59.2 parts water
	- Resulting ratio of gum in brine \bullet 1: 10,000
- * This is a mixed gum sold by Magnus, Maybee and Reynard, Inc. for making spice oil emulsions.

d. Gun karaya 1 part gum 2 parts oil 40 parts water Resulting ratio of gum in brine $\frac{1}{5}$ 1: 6000 e. Gun loccust bean 1 part gun 2 parts oil 40 parts water Resulting ratio of gum in brine $= 1$: 6000 7. Ratio of tweens and salt base emulsifiers. a. Tween 20 emulsifier 4 parts tween 20 1 part oil 4 parts water Resulting ratio of tween in brine ≈ 1 : 750 b. Tween 80 emulsifier 3 parts tween 80 1 part oil 4 parts water Resulting ratio of tween in brine \bullet 1: 1000 c. Salt base emlsifier 0.0.3. ' Resulting ratio of salt emulsifier in brine \equiv 1: 104

1- 0.0.8. spices made by Wm. J. Stange Co.

For the chemical experimental work on cloudiness in brine, it was decided to make up brines and add various ingredients to determine if they caused cloudiness.

Other chemical factors studied as possible causes of cloudiness were as follows:

- 1. Pickles in brine. Ihe pickles were in a brine of 16 per cent or 60° salemeter. These pickles were desalted and placed in pint jars, after which a dill brine was added to cover them. The finished brine tested four per cent salt and eight grains acid.
- 2. Sugar in brine. A brine was made up to represent a standard sweet brine. Its composition was: two per cent salt, 22 grain acid and 35 Brix. Phote-1ometer readings were observed to determine if sugar caused cloudiness.
- 3. Examination of comercial Jars. Photelometer readings were taken of sweet, processed and fresh pasteurized pickle brines.

FACIORS TESTED

I. Bacteriological Tests

Eight quart jars of pasteurized dills were obtained which showed a very cloudy brine. There were obtained from Michigan,

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Georgia and Texas. Several additional Jars were added which contained a very slight precipitate to determine also if this precipitate was of bacterial origin.

When the brine was examined bacteriologically these eight jars of pasteurized pickles appeared to contain largely gram positive rods ranging from single to short and long chains.

The brines were plated on the three media previously described, and microscopic examination of colonies isolated on the various media indicated relatively pure cultures of one organism. A total of 12 colonies of the bacteria were isolated and purified by repeated plating. These bacteria were further indentified by *stains*, cultural, morphorological, and biochemical characteristics. These tests placed them in the family Lactobactericoeae.

The two pickle jars containing a very slight precipitate showed a total count of 100 colonies per ml. Plates from these two jars showed that these colonies were gram positive, sporogenic, rod shaped bacteria. Since in these cases the total count did not indicate any great amount of sediment due to organisms, these organisms were not further indentified.

Biochemical studies and other characteristics indicated that the 12 organisms could be divided into two types. Salt, acid and pH were determined on the various brines. The results of these experiments are found in tables 1, 2 and 3.,

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Discussion of Results

The two quart jars which showed only slight sediment gave only 100 organisms per ml. The other jars which were very cloudy showed large numbers of bacteria present which caused the cloudy of the contents
brine. Chemical studies, of the various jars are shown in table 1 showed that the eight jars which had cloudy brine had also increased acid, calculated as acetic acid which was undoubtly due to the lactic acid-producing organisms present in the brines.

Biochemical studies and the morphological and cultural characteristics of the 12 organisms isolated indicated that they belonged to the genus, Lactobacillug and to two species plantarum and fermenti.

Etchells and Jones (10) and Fabian and Orloff (11) have shown that proper pasteurization carried out at 165° F for 15 minutes has been adequate to kill bacteria, yeasts and molds which would ferment the pickles. The survival of lactobacilli in these jars showed that these dills were not properly pasteurized at the recommended time and temperature. This resulted in a Etchells and Jones (10) and Fabian and Orloff (11) have
shown that proper pasteurisation carried out at 1650 F for
15 minutes has been adequate to kill bacteria, yeasts and molds
which would ferment the pickles. The surviv fermentation of the pickles by organisms of the genus Lactobacillus.

11. Physical Experiment

The effect of heat on gum emulsions and other oil emulsifiers.

This experiment was set up to determine if heat caused cloudiness in brines. The brines tested had the same composition as those listed under 111 Chemical studies. The results are given in table 4.

Discussion of Results

In this experiment a sediment oocured in all the gum emulsions in both the processed and the pasteurized brines. Although the vegetable gums acted as a stabilizer of the emulsion, the electrolytic effect of the solution may have caused precipitation of the gums. Results in table 4 showed that, the brines heated at 165° F for 15 minutes resulted in increased cloudiness in the brine. This was doubtless due to the combined effect of heat on the vegetable emulsifier and the electrolyte effect.

Tests on the precipitate to determine if it was carbohydrate in nature, by using the **Mollish test indicated that the** precipitate was a carbohydrate.

The results of salt base emulsifier (C.O.S.) and the tween emulsifier indicated that both served very well to disperse the oil into the brine, and what was more important neither one produced a precipitate in the brine.

> III. Chemical Experiments Effect of Whole and Powdered Spices

It has been known that the addition of whole and powdered spices causes increased sediment and sometimes cloudiness. Therefore, experiments wore set up to determine the factors involved.

In the Laboratory observations of the large Montana mustard seed, showed a gelatinous membrane being formed around the seeds

when they were placed in both fresh pasteurized and processed dill brines. This membrane could be broken by continued jaring and shaking and resulted in a gelatinous precipitate in these brines. When the Montana mustard seeds were placed in water and observed after a few days, the membrane extended its size to form a membrane of almost double the size observed in brines. Mustard seed placed in tap water and distilled water gave a gelatinous membrane. There was more formed in tap water than in distilled water.

A great variety of ground and whole spices were used, some of which were known to produce cloudiness in brines, such as powdered onion and garlic. These data are found in tables 5 and 6.

Discussion of Results

Results in table 5 indicate that powdered spices added to dill pickle brines, resulted in marked cloudiness as compared with the flakes, buds and oils of the same spices. Dehydrated garlic flakes gave excellent results, with a very slight reduction in photelometer readings.

in combination with various gums in most cases
In table 6 whole spices gave increased turbidity throughout. Microscopic examination of this sediment indicated that the sediment was amorphorous. Mo oil globules were present in the sediment. test
Million's research for the protein test gave positive results. Examination of the gelatinous precipitate obtained from soaking mustard seeds in tap and distilled water, showed the precipitate

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to be amorphorous and it gave a positive Million's test which indicated a protein.

Assuming that this protein was globulin in nature, investigation of its globulin characteristics and comparison with the protein precipitated in brine solution, showed the two to be similiar. Globulin protein is soluble in dilute salt solutions and insoluble in pure water. Mustard seeds placed in tap water showed a great amount of cloudiness, while those placed in distilled water did not show as much turbidity.

There was not as much cloudiness in brine solution as there was in water. In this case the globudin protein precipitated in a dilute salt'which may be considered a peptization process, and upon further increase of salt concentration, throughout a certain range, altered the surface forces in such a manner as to favor dispersion. Further increasing the salt concentration caused dehydration or the salting out of the protein micelles. Therefore, in the brine, it is possible that the salt concentration is in a peptization process. Furthermore tap water used had a pH $= 7$, while the fresh pasteurized dill brine had a pH $= 4$. This would result in the possibility that p H = 4 was not near the iso-olectric point of the protein fraction and, therefore, resulted in less precipitation.

However, results showed that the whole spices do cause cloudiness in brine, and that the brine serves chemically to increase this cloudiness depending cn the salt concentration and the pH.

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Effect of Water Hardness

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Believing that the various hardness
Believing that the various hardness of ed in many localities might result in cloudiness, tests were run using college water and distilled water. A typical analysis of Michigan State College water which is conditioned for laundry and power plant boilers is as follows:

In addition the water contained Crenothrix, Leptothrix and other iron bacteria.

In these experiments tests were run using tap water $(M.S.C.$ water of the above composition) and distilled water. Photelometer

readings of both tap water and distilled water were 100, therefore tests were made to determine differences in readings using acid alone, salt alone, and a regular processed brine. Various mustard seeds and gum emulsions were also used to determine effect of the waters on them in the presence of acid salt and processed brine. Results are shown in table 7.

Discussion of Results

In table 7 the results indicated that water hardness contributed to the cloudiness in dill pickle brines. There was not as great varation in readings by the photelometer when using tap or distilled water. However, when acid or salt was added to tap or distilled water cloudiness oecured. Since the cloudiness was greater with tap water, this indicated that the high mineral content of the tap water was either salted out or the acid reacted with the various anicns or cations present in the water which resulted in a cloudy brine.

Salt when added to different kinds of mustard seeds in tap or distilled water, at $p \mathbb{H}$ \mathbb{F} 7, resulted in a salting out of the protein portion of the mustard seeds. acted with the various anions or cations present in the water
which resulted in a cloudy brine.
Salt when added to different kinds of mustard seeds in
tap or distilled water, at pH = 7, resulted in a salting out
protein po

Aeid when added to different kinds of mustard seeds also resulted in increased turbidity due possibly to the effect of the protein of the various mustard seeds was at a pH near the isoelectric point.

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 $\mathcal{L}^{\mathcal{L}}$. We see that the contribution of the contrib $\label{eq:2.1} \mathcal{L}_{\mathcal{A}}(\mathcal{A}) = \mathcal{L}_{\mathcal{A}}(\mathcal{A}) = \mathcal{L}_{\mathcal{A}}(\mathcal{A}) = \mathcal{L}_{\mathcal{A}}(\mathcal{A})$ $\label{eq:2.1} \mathcal{L}(\mathcal{L}^{\text{max}}_{\mathcal{L}}(\mathcal{L}^{\text{max}}_{\mathcal{L}}(\mathcal{L}^{\text{max}}_{\mathcal{L}}(\mathcal{L}^{\text{max}}_{\mathcal{L}^{\text{max}}_{\mathcal{L}}(\mathcal{L}^{\text{max}}_{\mathcal{L}^{\text{max}}_{\mathcal{L}^{\text{max}}_{\mathcal{L}^{\text{max}}_{\mathcal{L}^{\text{max}}_{\mathcal{L}^{\text{max}}_{\mathcal{L}^{\text{max}}_{\mathcal{L}^{\text{max}}_{\mathcal{L}^{\text{max}}$ $\label{eq:2.1} \mathcal{L}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal$ $\mathcal{L}(\mathcal{L})$. The contract of the state $\mathcal{L}(\mathcal{L})$ $\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L$ $\label{eq:2.1} \mathcal{L}(\mathbf{r},\mathbf{r})=\frac{1}{2}\sum_{i=1}^n\mathcal{L}(\mathbf{r},\mathbf{r})\mathcal{L}(\mathbf{r},\mathbf{r})\mathcal{L}(\mathbf{r},\mathbf{r})\mathcal{L}(\mathbf{r},\mathbf{r})\mathcal{L}(\mathbf{r},\mathbf{r})\mathcal{L}(\mathbf{r},\mathbf{r})\mathcal{L}(\mathbf{r},\mathbf{r})\mathcal{L}(\mathbf{r},\mathbf{r},\mathbf{r})\mathcal{L}(\mathbf{r},\mathbf{r},\mathbf{r},\mathbf{$ $\label{eq:2.1} \mathcal{L}(\mathcal{L}^{\mathcal{A}}(\mathcal{L}^{\mathcal{A}}(\mathcal{L}^{\mathcal{A}}(\mathcal{L}^{\mathcal{A}}(\mathcal{L}^{\mathcal{A}}(\mathcal{L}^{\mathcal{A}}(\mathcal{L}^{\mathcal{A}}(\mathcal{L}^{\mathcal{A}}(\mathcal{L}^{\mathcal{A}}(\mathcal{L}^{\mathcal{A}}(\mathcal{L}^{\mathcal{A}}(\mathcal{L}^{\mathcal{A}}(\mathcal{L}^{\mathcal{A}}(\mathcal{L}^{\mathcal{A}}(\mathcal{L}^{\mathcal{A}}(\mathcal{L}^{\mathcal$ $\frac{1}{2} \sum_{i=1}^n \frac{1}{2} \sum_{j=1}^n \frac{1}{2} \sum_{j=$ $\mathcal{L}^{\mathcal{A}}$, and the set of t

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 $\frac{1}{2} \int_{\mathbb{R}^3} \left| \nabla \phi \right|^2 \, d\mathcal{H}^2 \$ the contract of $\mathcal{L}_{\mathcal{A}}$ and $\mathcal{L}_{\mathcal{A}}$ are the set of the set of the following \mathcal{A} . As follows: $\mathcal{L}(\mathcal{L}(\mathcal{L}))$ and $\mathcal{L}(\mathcal{L}(\mathcal{L}))$. The contribution of $\mathcal{L}(\mathcal{L})$ $\label{eq:2.1} \mathcal{L}(\mathcal{L}^{\mathcal{A}}(\mathcal{A})) = \mathcal{L}(\mathcal{L}^{\mathcal{A}}(\mathcal{A})) = \mathcal{L}(\mathcal{L}^{\mathcal{A}}(\mathcal{A})) = \mathcal{L}(\mathcal{L}^{\mathcal{A}}(\mathcal{A}))$

When acid, salt and whole spices were used together, the photolometer readings indicated that the various constituents apparently buffered each other and cloudiness was not as low as when either salt or acid was used seperately. The decreased cloudiness may have resulted because the pH was not near the isoelectric point or that the solubility of the protein was in the peptization process.

Results of this experiment, however, indicated that the acid and salt precipitated out various minerals in tap water, and this resulted in higher cloudiness in pickle brines.

Effect of Salt

Salt as a constituent of brines should be of high purity. In this work granulated salt was used which was of the same grade comonly used in the pickle industry. Although the effect of salt on whole spices $w_B s$ recorded in table 7 this work showed the effect of salt on various other brine constituents. Salt brine of four per cent salt concentration was placed in pint jars, sealed and examined after one week. These results are shown in table 8.

Discussion of Results

The results in table 8, indicated that a salt brine of four per cent, has a cloudiness due to the action of the salt on the anions and cations present in tap water. Results also showed that

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the salt will "salt out" the protein fractions as well as precipitate out a portion of the vegetable gum. Photelometer readings obtained from Brassica alba and Brassica niger are very low. This can be accounted for in that these mustard seeds are crushed; therefore, the brine contains many small particles of these seeds.

Effect of Acid

Vinegar as used in brines is commonly bought in tank cars in concentrated form. It is diluted to the desired concentration depending on the type of pickle product being processed. In this work the acid was diluted to eight grains acid with tap water. various spices were also added to determine the effect of acid on them. The photolometer readings were observed after one week and are recorded in table 9.

Discussion of Results

In table 9, the results indicated that vinegar caused ^a slight turbidity in tap water. This is due to the effect of the acid on the minerals present in the water. The increased cloudinose with acid and whole spices, may be due in part to the action of the acid on the water, or the cations and anions present causing a precipitation with the mustard protein. The effect of acid on the gum emulsions indicated that a portion of the gum precipitates out.

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Effect of Acid and Salt

In this experiment it was decided to determine if gum emulsions added to tap and distilled water would cause a cloudiness. The gun emulsions were added one part emulsions to 3000 parts water. The effect of acid and salt were also studied by making up a brine solution of eight grain acid and four per cent salt. Various ingredients were added to this brine to determine the combined effect. The results are shown in table 10.

Discussion of Results

These results showed that the acid and salt were not wholly responsible for increased turbidity, but that many factors may cause cloudiness. Results showed that the gum emulsions, when added to tap water and distilled water, were precipitated out.

Distilled water gave only slightly higher results which would indicate that a certain amount of gum is precipitated as the oil is dispersed throughout the brine. It may also indicate that the vegetable gum which acts as an emulsion stabilizer was present in excess. However, results as shown here and in table 6 do indicate that the acid and salt do cause protein precipitation which results in increased cloudiness.

Effect of Alum

Alum is used extensively in the pickle industry to crisp pickles, especially processed and genuine dills. Alum is added

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to the brine directly or may be added in the desalting of the salt stock pickles by allowing the pickles to soak in an alum solution. The amount of alum $\text{Al}_2(\text{SO}_4)_5$ 18 H₂O, used was sufficient to give a final concentration of 0.1 per cent by weight in the brine.

Photelometer readings were also made with alum and a buffered solution. The same concentration of alum was used as with brine solutions. Readings showed a large precipitation at pH 5.5 and up into the alkaline range with the greatest cloudiness at pH 6.

The results using brines composed of various spices and gum emulsions with alum added are shown in table 11.

Discussion of Results

The experimental work as recorded in table 11 showed that alum is definitely precipitated in dill pickle brines and resulted in increased cloudiness. Further evidence was accumulated in work done on genuine dills. The genuine dills can be packaged in the brine in which they have fermented or they can be packaged in a new brine. The fermented brine was very cloudy due to the fermentation. Genuine dill pickles were packaged in four various ways as follows;

- 1. Filtered the brine in a filter cell and placed this brine on the dills.
- 2. Placed non-filtered brine on the dill pickles.
- 3. Filtered the brine and then added alum.
- 4. Alum added to the brine unfiltered.

-21-

The results showed that the alum was precipitated after the genuine dills had been pasteurized, as shown in the following:

- 1. Brine was very clear, with no precipitate or sediment.
- 2. Brine was very cloudy, with precipitate on bottom and on pickles.
- 3. Brine was fairly clear, however a floculant precipitate was observed on the bottom of the jar.
- 4. Brine was very cloudy with precipitate on pickles and on the bottom of the jar.

The precipitate did not give a positive Mollish or Million's test and, therefore, it may be assumed that the alum itself precipitated from, the genuine dill brine, upon pasteurizing.

Effect of Pickles on Cloudiness

A.study was made of the effect of the pickles themselves causing the cloudiness.

The pickles used were salt stock pickles in a brine of 16 per cent salt or 60° salometer. The pickles were desalted and placed in pint jars, after which a dill brine was added to cover them. The finished brine tested four per cent salt and eight grain acid. various spices were added to similate actual conditions of packaging. The results are given in table 12.

Discussion of Results

The results in table 12 showed that in this experiment the pickles did not cause extensive cloudiness of the brine. Un-

doubtdy any pickle which was not washed properly will show sediment. Microscopic examination of this sediment did not show any epithelial cells to be the cause of turbidity.

In other experimental work on the cloudiness of pickles in brine, various dill and sweet pickles from commercial companys 'were examined for turbidity by the photolometer. These results are given in table 13.

Discussion of Results

Examination of commercial packs by means of the photolometer showed that the turbidity was evident in all dill and sweet pickles. The readings as given in table 13 were all acceptable from the sales standpoint, however turbidity is evident in all the packs. It would seem that photelometer readings below 80 would become objectionable.

Effect of Sugar

Many examinations of high, normal and low brix sweet pickles, from the grocers shelves, have shown little or no cloudiness, with no sediment. In this experiment the brine was made up to have two per cent salt, 22 grain acid and a Brix of 35. This brine was added to pint jars, sealed and observed after one week. The results are shown in table 14.

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Discussion of Results

The results showed that sweet pickles also may have a cloudy brine. It we probable that the sugar holds the various particles of sediment in suspension, as pectin does in jellies. Most sweet pickles are packed in small jars, and due to the smaller volume of brine, and to the better transmission of light, they do not appear cloudy. However, care should be used in the ingredients used such as emulsion gums, powdered spices, and the whole spices.

Summary

Processed and fresh pasteurized dill pickle brines, simulating Summary
Processed and fresh pasteurized dill pickle brines, simul
examiestly commercial brines, were examined bacteriologically, physically and chemically to determine the various factors causing cloudiness in them.

Samples of cloudy commercial pasteurized dills were examined bacteriologically and found to have undergone a lactic acid fermentation. Isolation and study of the predominant bacteria, showed them to be, Lactobacillus and that they belonged to the al brines, we
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Lactobacillus species plantarum and fermenti. It is well known that proper pasteurization at 165° for 15 minutes is sufficient to kill all yeast and bacteria, except spore forming bacteria, which gradually die out.

Vegetable gum emulsions were shown to cause turbidity in dill brines, due to the action of temperature, electrolytes and instability of gum emulsions in hard waters.

Salt b_6 ses ($C.0.8.$) and Tween 20 and 80 which act as essential oil carriers were shown to cause little or no cloudiness in dill brines.

The addition of whole spices to dill brines caused cloudiness. Montana mustard seed gave considerably more gelatenous precipitate than did the other two, Orientia and Superior seed, tested.

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Tests made with tap water and hard water indicated that the various minerals present, as cations and anions in hard water, increased the cloudiness in the presence of acid and salt.

Alum.caused cloudiness in dill pickle brines. This action may be due to increasing the electrolyte acitivity of the brine.

Tests on sugar brines indicated that various sugar brines may also be cloudy as judged by the photolometer, but often the particles remain suspended and therefore are not seen in the small bottles.

There are many factors which cause cloudiness. Chemically it may involve pH, electrolytes, iso-electric points, anions and cations, which acting together or separately may cause cloudiness.

Physically heat causes cloudiness in certain brines by coagulation and precipitation of certain substances such as proteins.

Bacteriologically cloudiness is due to the growth of certain acid tolerant bacteria.

Conclusion

The factors responsible for cloudiness in pasteurized and processed dill brines may be listed as:

- 1. Microorganisms in improperly pasteurized dills.
- 2. Vegetable gum emulsions
- 3. Whole spices.
- 4. Powdered spices
- 5. Alum
- 6. Hard'water
- 7. Vinegar under certain conditions
- 8. Salt under certain conditions

Table l. - The chemical analysis of pasteurized dills containing a cloudy brine due to bacteria.

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Table 2. - Cultural characteristics of 12 cultures of microaerophilic bacteria isolated from cloudy brine of pasteurized dills. \mathbb{R}^2

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Table 3. - Biochemical characteristics of 12 cultures of microaerophilic b_acteria isolated from cloudy brine of pasteurized dills.

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Table 4. - The effect of heat or gum emulsions and other oil carring bases causing cloudiness in brine. Thble 4. - The effect of heat or gum emulsions and other oil carring bases causing cloudiness in brine.

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Table 5. - The effect of powdered spices on cloudiness in processed dill brine when used with various emulsifying gums. 5. - The ef
brine
Spice added

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Table 6. - The effect of whole spices on cloudiness of fresh pasteurized dill brine when used with various emulsifying agents.

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Table 8. $-$ The effect of salt in combination with various spices and a mixed gum in causing cloudiness in four per cent - The effect of salt
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Table 9. - The effect of vinegar in combination with whole spices and gum emulsion in causing cloudiness in eight grain acid brine. -36-

The effect of vinegar in combination with whole

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Photelometer

Composition of brine

reading

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 $\label{eq:2.1} \frac{1}{\sqrt{2}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2.$ $\label{eq:2.1} \mathcal{L}(\mathcal{L}^{\text{max}}_{\mathcal{L}}(\mathcal{L}^{\text{max}}_{\mathcal{L}}),\mathcal{L}^{\text{max}}_{\mathcal{L}}(\mathcal{L}^{\text{max}}_{\mathcal{L}}))$

 $\label{eq:2.1} \mathcal{L}(\mathcal{L}^{\mathcal{L}}_{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}_{\mathcal{L}})) = \mathcal{L}(\mathcal{L}^{\mathcal{L}}_{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}_{\mathcal{L}})) = \mathcal{L}(\mathcal{L}^{\mathcal{L}}_{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}_{\mathcal{L}})) = \mathcal{L}(\mathcal{L}^{\mathcal{L}}_{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}_{\mathcal{L}}))$ $\mathcal{L}^{\text{max}}_{\text{max}}$ and $\mathcal{L}^{\text{max}}_{\text{max}}$

 $\label{eq:2} \frac{1}{\sqrt{2}}\int_{0}^{2\pi} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^$ $\mathcal{L}^{\text{max}}_{\text{max}}$ and $\mathcal{L}^{\text{max}}_{\text{max}}$

 $\mathcal{L} = \{1, \ldots, n\}$. $\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L$

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 $\label{eq:2.1} \mathcal{L}^{\mathcal{A}}(\mathcal{A})=\mathcal{L}^{\mathcal{A}}(\mathcal{A})\otimes\mathcal{L}^{\mathcal{A}}(\mathcal{A})\otimes\mathcal{L}^{\mathcal{A}}(\mathcal{A})\otimes\mathcal{L}^{\mathcal{A}}(\mathcal{A})\otimes\mathcal{L}^{\mathcal{A}}(\mathcal{A})\otimes\mathcal{L}^{\mathcal{A}}(\mathcal{A})\otimes\mathcal{L}^{\mathcal{A}}(\mathcal{A})\otimes\mathcal{L}^{\mathcal{A}}(\mathcal{A})\otimes\mathcal{L}^{\mathcal{A}}(\mathcal$

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 $\label{eq:2.1} \mathcal{L}(\mathcal{L}) = \mathcal{L}(\mathcal{L}) = \mathcal{L}(\mathcal{L}) = \mathcal{L}(\mathcal{L}) = \frac{1}{\mathcal{L}} \sum_{i=1}^{\mathcal{L}} \mathcal{L}(\mathcal{L}) \mathcal{L}(\mathcal{L}) = \mathcal{L}(\mathcal{L})$

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 $\label{eq:2.1} \mathcal{L}(\mathcal{L}^{\mathcal{L}}_{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}_{\mathcal{L}})) = \mathcal{L}(\mathcal{L}^{\mathcal{L}}_{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}_{\mathcal{L}})) = \mathcal{L}(\mathcal{L}^{\mathcal{L}}_{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}_{\mathcal{L}}))$

Ihble 10. - the effect of acid and salt in combination with other brine constituents in causing cloudiness e 10. - The effect of acid and salt in combination with

ether brine constituents in causing cloudiness

Composition of brine

Photelometer reading

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 $\sim 10^{-11}$

 $\sim 10^{-1}$

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 $\label{eq:2.1} \frac{1}{\sqrt{2}}\int_{0}^{\infty}\frac{1}{\sqrt{2\pi}}\left(\frac{1}{\sqrt{2\pi}}\right)^{2\alpha} \frac{1}{\sqrt{2\pi}}\int_{0}^{\infty}\frac{1}{\sqrt{2\pi}}\left(\frac{1}{\sqrt{2\pi}}\right)^{\alpha} \frac{1}{\sqrt{2\pi}}\int_{0}^{\infty}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}$

 $\label{eq:2.1} \frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1$

Table 11. - The effect of alum in combination with spices and gums in causing cloudy pickle brine. $\sim 10^{-1}$

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 $\label{eq:2.1} \begin{split} \mathcal{L}^{(1)}(z) &= \mathcal{L}^{(1)}(z) + \mathcal{L}^{(1)}(z) + \mathcal{L}^{(2)}(z) + \mathcal{L}^{(3)}(z) \\ &= \mathcal{L}^{(1)}(z) + \mathcal{L}^{(2)}(z) + \mathcal{L}^{(3)}(z) + \mathcal{L}^{(4)}(z) + \mathcal{L}^{(5)}(z) \end{split}$

 $\label{eq:2.1} \mathcal{L}(\mathcal{L}(\mathcal{L})) = \mathcal{L}(\mathcal{L}(\mathcal{L})) = \mathcal{L}(\mathcal{L}(\mathcal{L})) = \mathcal{L}(\mathcal{L}(\mathcal{L})) = \mathcal{L}(\mathcal{L}(\mathcal{L})) = \mathcal{L}(\mathcal{L}(\mathcal{L}))$ $\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}})$ and $\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}})$. The contribution of the contribution of $\mathcal{L}^{\mathcal{L}}$ $\label{eq:2.1} \mathcal{L}(\mathcal{L}) = \mathcal{L}(\mathcal{L}) \mathcal{L}(\mathcal{L}) = \mathcal{L}(\mathcal{L}) \mathcal{L}(\mathcal{L})$ $\mathcal{L}^{\mathcal{A}}$ and the second constant $\mathcal{L}^{\mathcal{A}}$ are the second constant of $\mathcal{L}^{\mathcal{A}}$ $\label{eq:2.1} \mathcal{L}_{\mathcal{A}}(\mathcal{A})=\mathcal{L}_{\mathcal{A}}(\mathcal{A})=\mathcal{L}_{\mathcal{A}}(\mathcal{A})=\mathcal{L}_{\mathcal{A}}(\mathcal{A})=\mathcal{L}_{\mathcal{A}}(\mathcal{A})=\mathcal{L}_{\mathcal{A}}(\mathcal{A})=\mathcal{L}_{\mathcal{A}}(\mathcal{A})=\mathcal{L}_{\mathcal{A}}(\mathcal{A})=\mathcal{L}_{\mathcal{A}}(\mathcal{A})=\mathcal{L}_{\mathcal{A}}(\mathcal{A})=\mathcal{L}_{\mathcal{A}}(\mathcal{A})=\mathcal{L}_{\mathcal{A}}(\$ $\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L$ $\mathbf{C}_{\text{intra}}(\mathbf{r},\mathbf{r}) = \mathbf{C}_{\text{intra}}(\mathbf{r},\mathbf{r}) = \mathbf{C}_{\text{intra}}(\mathbf{r},\mathbf{r}) = \mathbf{C}_{\text{intra}}(\mathbf{r},\mathbf{r}) = \mathbf{C}_{\text{intra}}(\mathbf{r},\mathbf{r})$ $\mathcal{L}^{\mathcal{L}}$, where $\mathcal{L}^{\mathcal{L}}$ is the contract of the final $\mathcal{L}^{\mathcal{L}}$ $\mathcal{L}^{\text{max}}_{\text{max}}$ and $\mathcal{L}^{\text{max}}_{\text{max}}$ $\label{eq:2.1} \mathcal{L}_{\mathcal{A}}(\mathcal{A}) = \mathcal{L}_{\mathcal{A}}(\mathcal{A}) + \mathcal{L}_{\mathcal{A}}(\mathcal{A}) + \mathcal{L}_{\mathcal{A}}(\mathcal{A}) + \mathcal{L}_{\mathcal{A}}(\mathcal{A})$ $\label{eq:2.1} \frac{1}{\sqrt{2}}\int_{0}^{\infty}\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2}d\mu\int_{0}^{\infty}\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2}d\mu\int_{0}^{\infty}\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2}d\mu\int_{0}^{\infty}\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2}d\mu\int_{0}^{\infty}\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2}d\mu\$ $\label{eq:2.1} \mathcal{L}(\mathcal{A})=\mathcal{L}(\mathcal{A})\otimes\mathcal{L}(\mathcal{A})\otimes\mathcal{L}(\mathcal{A})\otimes\mathcal{L}(\mathcal{A})\otimes\mathcal{L}(\mathcal{A})\otimes\mathcal{L}(\mathcal{A})\otimes\mathcal{L}(\mathcal{A})\otimes\mathcal{L}(\mathcal{A})\otimes\mathcal{L}(\mathcal{A})\otimes\mathcal{L}(\mathcal{A})\otimes\mathcal{L}(\mathcal{A})\otimes\mathcal{L}(\mathcal{A})\otimes\mathcal{L}(\mathcal{A})\otimes\mathcal{$ $\ddot{}$ $\mathcal{L}^{\text{max}}_{\text{max}}$, where $\mathcal{L}^{\text{max}}_{\text{max}}$ $\mathcal{L}^{\text{max}}_{\text{max}}$ $\mathcal{L}^{\mathcal{L}}$ and the following the set of $\label{eq:2.1} \mathcal{L}^{\mathcal{A}}_{\mathcal{A}}(\mathcal{A})=\mathcal{L}^{\mathcal{A}}_{\mathcal{A}}(\mathcal{A})=\mathcal{L}^{\mathcal{A}}_{\mathcal{A}}(\mathcal{A})=\mathcal{L}^{\mathcal{A}}_{\mathcal{A}}(\mathcal{A})=\mathcal{L}^{\mathcal{A}}_{\mathcal{A}}(\mathcal{A})=\mathcal{L}^{\mathcal{A}}_{\mathcal{A}}(\mathcal{A})$ $\label{eq:2.1} \mathcal{L}_{\mathcal{A}}(\mathcal{A})=\mathcal{L}_{\mathcal{A}}(\mathcal{A})\otimes\mathcal{L}_{\mathcal{A}}(\mathcal{A})\otimes\mathcal{L}_{\mathcal{A}}(\mathcal{A})\otimes\mathcal{L}_{\mathcal{A}}(\mathcal{A})\otimes\mathcal{L}_{\mathcal{A}}(\mathcal{A})\otimes\mathcal{L}_{\mathcal{A}}(\mathcal{A})\otimes\mathcal{L}_{\mathcal{A}}(\mathcal{A})\otimes\mathcal{L}_{\mathcal{A}}(\mathcal{A})\otimes\mathcal{L}_{\mathcal{A}}(\mathcal{A})\otimes\mathcal{$

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 $\mathcal{L}^{\mathcal{A}}(\mathcal{A})$ and $\mathcal{A}^{\mathcal{A}}(\mathcal{A})$ are the set of the following $\mathcal{A}^{\mathcal{A}}(\mathcal{A})$. In the following $\mathcal{A}^{\mathcal{A}}(\mathcal{A})$ $\label{eq:2.1} \frac{1}{\sqrt{2}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2\left(\frac{1}{\sqrt{2}}\right)^2\left(\frac{1}{\sqrt{2}}\right)^2\left(\frac{1}{\sqrt{2}}\right)^2\left(\frac{1}{\sqrt{2}}\right)^2\left(\frac{1}{\sqrt{2}}\right)^2.$

 $\label{eq:3.1} \mathcal{L}(\mathcal{L}^{\text{c}}(\mathcal{L}^{\text{c}})) = \mathcal{L}(\mathcal{L}^{\text{c}}(\mathcal{L}^{\text{c}})) = \mathcal{L}(\mathcal{L}^{\text{c}}(\mathcal{L}^{\text{c}})) = \mathcal{L}(\mathcal{L}^{\text{c}}(\mathcal{L}^{\text{c}})) = \mathcal{L}(\mathcal{L}^{\text{c}}(\mathcal{L}^{\text{c}})) = \mathcal{L}(\mathcal{L}^{\text{c}}(\mathcal{L}^{\text{c}})) = \mathcal{L}(\mathcal{L}^{\text{c}}(\$ $\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}})$ and $\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}})$ and $\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}})$ and $\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}})$

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 $\label{eq:2.1} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^{2} \left(\frac{1}{\sqrt{2}}\right)^{2} \left(\$ $\label{eq:2.1} \frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2\pi}}\int_{\mathbb{R}^3}\frac{1$ $\label{eq:2.1} \mathbf{C} = \left\{ \begin{array}{ll} \mathbf{C} & \mathbf{C} & \mathbf{C} \\ \mathbf{C} & \mathbf{C} & \mathbf{C} \end{array} \right. \quad \text{and} \quad \mathbf{C} = \left\{ \begin{array}{ll} \mathbf{C} & \mathbf{C} & \mathbf{C} \\ \mathbf{C} & \mathbf{C} & \mathbf{C} \end{array} \right. \quad \text{and} \quad \mathbf{C} = \left\{ \begin{array}{ll} \mathbf{C} & \mathbf{C} & \mathbf{C} \\ \mathbf{C} & \mathbf{C} & \mathbf{C}$ $\label{eq:2.1} \mathcal{L}^{\mathcal{A}}_{\mathcal{A}}=\mathcal{L}^{\mathcal{A}}_{\mathcal{A}}\left(\mathcal{A}^{\mathcal{A}}_{\mathcal{A}}\right)=\mathcal{L}^{\mathcal{A}}_{\mathcal{A}}\left(\mathcal{A}^{\mathcal{A}}_{\mathcal{A}}\right)=\mathcal{L}^{\mathcal{A}}_{\mathcal{A}}\left(\mathcal{A}^{\mathcal{A}}_{\mathcal{A}}\right)=\mathcal{L}^{\mathcal{A}}_{\mathcal{A}}\left(\mathcal{A}^{\mathcal{A}}_{\mathcal{A}}\right)=\mathcal{L}^{\mathcal{$

 $\label{eq:2} \frac{1}{\sqrt{2}}\int_{\mathbb{R}^{2}}\left(\frac{1}{\sqrt{2}}\right)^{2}e^{-\frac{1}{2}\left(\frac{1}{2}\right)^{2}}\left(\frac{1}{\sqrt{2}}\right)^{2}e^{-\frac{1}{2}\left(\frac{1}{2}\right)^{2}}\left(\frac{1}{2}\right)^{2}e^{-\frac{1}{2}\left(\frac{1}{2}\right)^{2}}\left(\frac{1}{2}\right)^{2}e^{-\frac{1}{2}\left(\frac{1}{2}\right)^{2}}\left(\frac{1}{2}\right)^{2}e^{-\frac{1}{2}\left(\frac{1}{2}\right)^{2}}\left(\$

 $\label{eq:2.1} \frac{1}{\sqrt{2}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2\left(\frac{1}{\sqrt{2}}\right)^2\left(\frac{1}{\sqrt{2}}\right)^2\left(\frac{1}{\sqrt{2}}\right)^2\left(\frac{1}{\sqrt{2}}\right)^2\left(\frac{1}{\sqrt{2}}\right)^2\left(\frac{1}{\sqrt{2}}\right)^2.$

 $\label{eq:2.1} \frac{1}{\sqrt{2}}\int_{0}^{\infty}\frac{1}{\sqrt{2\pi}}\left(\frac{1}{\sqrt{2\pi}}\right)^{2\alpha} \frac{1}{\sqrt{2\pi}}\int_{0}^{\infty}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{$ $\label{eq:2.1} \mathcal{L}(\mathcal{L}(\mathcal{L})) = \mathcal{L}(\mathcal{L}(\mathcal{L})) = \mathcal{L}(\mathcal{L}(\mathcal{L})) = \mathcal{L}(\mathcal{L}(\mathcal{L})) = \mathcal{L}(\mathcal{L}(\mathcal{L}))$

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 $\label{eq:2.1} \frac{1}{\sqrt{2}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2.$

 $\label{eq:2.1} \frac{1}{\sqrt{2}}\int_{\mathbb{R}^3}\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2.$

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