ABSTRACT

FRANKOWSKI, KRISTIN M. The Role of Sodium in the Salty Taste of Permeate. (Under the direction of Dr. Maryanne Drake).

One of the contributing factors to hypertension in the U.S. is from the amount of sodium in the American diet. Many food companies are trying to limit the amount of sodium in their products. Permeate, the liquid remaining after whey or milk is ultrafiltered, has been suggested as a salt substitute. It has not been established what part of permeates composition allows for its use as a sodium replacer or if permeates exhibit salty taste. The objective of this study was to determine the sensory and compositional properties of permeates and to determine if elements other than sodium (Na) contribute to the salty taste found in permeate. Eighteen whey (n=14) and reduced lactose (n=4) permeates were obtained in duplicate from commercial facilities. Proximate analyses, specific mineral content, and non protein nitrogen were determined. Organic acids and nucleotides were extracted followed by high performance liquid chromatography (HPLC). Aromatic volatiles were evaluated by gas chromatography mass spectrometry (GCMS). Descriptive analysis of permeates and model solutions was conducted using a trained sensory panel. Correlation analysis was used to determine which compounds were likely associated with salty taste. Whey permeates were characterized by cooked/milky and brothy flavors, sweet taste and low salty taste. Permeates with lactose (DLC) removed were distinctly salty. The organic acids with the highest concentration in permeates were lactic and citric acid. Volatiles included aldehydes, sulfur containing compounds, and diacteyl. Sensory tests with sodium chloride solutions confirmed salty taste of DLC was not solely due to sodium present. Permeate models were created with

KCL, lactic acid, citric acid, hippuric acid, uric acid, orotic acid, and urea. KCl, lactic, and orotic acid were found to contribute to salty taste.

The Role of Sodium in the Salty Taste of Permeate

by
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DEDICATION

To my parents for their never-ending support.

BIOGRAPHY

Kristin Frankowski was born in Chicago, IL. In the fall of 2006 she began her undergrad studies at Loyola University Chicago. One year after, in the fall of 2007, she transferred to the University of Illinois Urbana Champaign. It was here that she found her love of food science. Kristin had always had an interest in chemistry but wasn't sure what she would want to pursue as a career. As an elective class she took an introduction to food science course and become more interested in that class than any of her others. It was after that course that she switched her major to food science. She graduated in December of 2012 with her Bachelors in Food science. In the fall of 2011, she began her Masters under the direction of Dr. Maryanne Drake.

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CHAPTER 1

Literature Review

Salt

Sodium chloride (NaCl), otherwise known as salt, has many uses throughout the food industry. Its uses are currently grouped into three major categories: processing, sensory, and preservation (Hutton et al., 2002). However, salt may function in more than one category at a time: overlapping between the categories is frequent. Sodium chloride is one of the oldest preservatives in the world; it was used mainly to preserve meats before the time of refrigeration.

Sodium Chloride

Not only is NaCl useful in the food industry, it is also needed by the body to perform certain functions. Sodium is an electrolyte and is used in the body to create electrical signals for communication in the brain, nervous system, and muscles. Sodium also regulates the amount of water that is in the body (BNF, 1994; Eck and Wilson, 1991; SACN, 2003). According to the Dietary Guidelines for Americans, the average person, ages 9-50 y, should have 1500- 2400 mg per day (USDA, 2010). However, most Americans consume too much sodium, with only 15% of the population at or below the recommended consumption (USDA, 2010). Most Americans consume in excess of the upper limit (2400 mg/day). The average sodium consumption in the US is around 3,000 mg per day: 55% more than five years ago (Harvard Medical, 2010).

The average sodium consumption by age is shown in Figure 1.1. Most of the salt consumed is in processed foods, not from salt that is added during home preparation or at the table (Anderson et al., 2009). In a study conducted on dietary sources of sodium in many regions (INTERMAP), the main sources of US sodium were: breads/grains/cereals (27% of dietary

sodium), commercially processed meats (12% dietary sodium), gravies/seasonings/sauces/salad dressing (11.7% dietary sodium), and dairy products (8.2% dietary sodium) (Anderson et al., 2009).

Adverse Health Effects

High sodium consumption is cause for concern because too much salt intake can have adverse health effects. Salt has negative effects on gastric health (Tsugane et al., 2004), kidney stones (Cirillo et al., 1994), asthma (Carey et al., 1993), and increases blood pressure. In rats, salt increased carcinogenic effects on known gastric carcinogens such as N-methyl-N-nitro-N-nitrosoguanidine (MNNG) (Tsugane et al., 2004). High concentrations of salt destroy the mucus lining in the stomach and can cause inflammation and other negative impacts (Tsugane et al., 2004). Sodium was also shown to increase the colonization of *H. pylori*, the bacteria responsible for ulcers, in mice (Fox et al., 1999). High Na: K ratios were also related to the formation of urinary stones in the bladder. These high Na: K ratios were correlated to the amount of dietary sodium consumed (Cirillo et al., 1994). High urinary Na is linked to high calcium excretion: which ultimately leads to the development of kidney stones (Cirillo et al., 1994).

Hypertension

The greatest health effect that a high intake of NaCl causes is its affect on blood pressure and ultimately hypertension. The body uses a rise in blood pressure to prevent sodium accumulation (Guyton, 1991). According to the United States Department of Health and Human Services, 65 million Americans suffer from high blood pressure. Another 59 million have "prehypertension" (United States Department of Health and Human Services, 2006).

Prehypertension is when a person's blood pressure is above normal but it is not yet elevated to hypertension levels. Hypertension is when blood pressure levels are above 140/mmHg, systolic blood pressure, and above 90/mmHg, diastolic blood pressure, (US Department of Health and Human Services, 2006). The probability of getting hypertension varies from person to person and within certain ethnic and age groups. Especially prone groups are African Americans and older Americans (Cutler et al., 2008; Giles et al., 2007; Lionaki et al., 2012). Also, some individuals have salt sensitivities. A salt sensitivity is a genetic phenotype which makes someone more likely to develop high blood pressure after long exposure to salt (Jianjun, 2009).

A series of studies, known as the INTERSALT studies, showed that a high, above the upper limit of 2400 mg/day, salt consumption was related to increases in blood pressure (Stamler, 1997). In this study, more than 10,000 subjects worldwide and 52 different communities were polled. From each community they tested the amount of sodium that was excreted in the urine and its relation to blood pressure. Some of the areas polled had low sodium excretion and low blood pressure. For example, in the Yanomamo Indians of Brazil the amount of sodium they excreted was 0.2 mmol/24 hours or 4.6mg /day (INTERSALT Cooperative Research Group, 1988). Other areas had as high as 242 mmol/24 hours or 5566 mg/day, such as in North China (INTERSALT Cooperative Research Group, 1988). This study also showed that blood pressure tended to increase with age. In the centers that had high salt intake there was a significant relationship between salt intake and the slope of blood pressure with age, but in areas with low salt intake and low sodium consumption this effect was not observed (INTERSALT Cooperative Research Group, 1988). INTERSALT studies

also found a relationship between the amount of potassium excreted in urine and blood pressure. A negative correlation was found between the intake of potassium and blood pressure (INTERSALT Cooperative Research Group, 1988).

Because of INTERSALT and many other studies linking high sodium intake to high blood pressure, the United States Department of Health and Human Services and other organizations have come up with a diet plan that helps to lower blood pressure and to ultimately reduce illnesses caused by hypertension. This diet plan is known as the Dietary Approaches to Stop Hypertension or the DASH diet plan (US Department of Health and human Services, 2006). The DASH diet plan is low in fats (both total and saturated) and cholesterol, while encouraging the consumption of fruits, vegetables, and low fat milk (US Department of Health and Human Services, 2006). The DASH diet reduces sodium intake, while encouraging the consumption of potassium and other minerals that have been shown to lower blood pressure (US Department of Health and Human Services, 2006). Table 1.1 outlines the guidelines for the DASH diet. Studies done on the DASH diet have shown significant reductions in blood pressure. Subjects that were on the DASH diet reduced their blood pressure by 11.2/7.5 mm Hg (Blumenthal et al., 2010). Although many studies have shown the correlation between sodium and high blood pressure, people are still hesitant about low sodium diets (FDA, 2008); 50% of consumers would like to decrease their sodium but don't want to lose the flavor of their foods (NMI, 2008).A study done by Kim et al. (2012) characterized consumer knowledge of sodium and sodium reduced food by surveying consumers. Consumers were aware of a link between certain medical conditions, like hypertension, and high sodium consumption. Even though consumers were aware of this

relationship, salt was considered a must have by consumers and they preferred a "reduced" sodium version of the food product compared to a sodium "free" product.

Satiety

Salt has been shown to cause a significant decline in the desire to eat after consumption of salty foods (Guinard et al., 1998). Appetite has two main components to it: satiation and satiety. Satiation is when to end the meal when eating, essentially when someone is full (Geraedts et al., 2010).This helps to prevent overconsumption (Cummings and Overduin, 2007). Satiety determines the amount of time between meals, or how often you eat (Geraedts et al., 2010). Satiety is controlled by both mechanical and hormonal stimulation (Karhunen et al., 2008). The major hormones that contribute to satiety are Ghrelin, Cholecystokinn (CCK), Glucagon- like peptide 1 (GLP-1), and Peptide YY (PYY) (Geraedts et al., 2010). These hormones are known as satiation signals; they work by limiting the amount of food intake, and are released at different areas within the GI tract (Geraedts et al., 2010).

Diet composition of macronutrients is known to affect satiation and satiety. A high protein diet is thought to have the greatest effect on increasing satiety (Geraedts et al., 2010; Sorerens, 2008; Veldhorst, 2008). However, recent studies have started to show that the five basic tastes (known as tastants) also contribute to satiety (Garaedts et al., 2011). It is generally known that there are certain receptors located on the tongue that detect tastants, but it is thought that these same receptors are located in the gut (Bezencon et al., 2007).Because of these receptors, the GI tract is able to sense the different tastes (Garaedts et al.,2011).

In a recent study, researchers demonstrated that NaCl had an effect on satiation and satiety by causing a release in hormonal signals (Garaedts et al., 2011). NaCl was shown to release

CCK and GLP-1 dose dependently; CCK and GLP- 1 reached their highest levels when NaCl was about 400mM (Garaedts et al., 2011). It was also shown that the release of CCK and GLP-1 by NaCl increased over time (Garaedts et al., 2011). This is important because if the salty tastant is lost in the reduction of sodium, then satiety will decrease. If other salts can replace sodium then the satiety can still be maintained in the food.

Physiology of Salty Taste

Taste buds on the tongue are made up of groups of taste receptor cells. The taste receptor cells are able to detect a wide range of chemicals by transforming chemical signals into a series of action potentials for nerve fibers (Bigiani et al., 2003). This is done by two general processes: transduction and coding (Gilbertson et al., 2000). Transduction is the process of turning the chemical stimulus into a chemical signal. Coding takes the signal produced from transduction and turns it into action potential to the nerves. The general mechanism for salt taste transduction is that the flow of cations, usually Na^+ though other ions as well (K^+, Li^+) produce membrane depolarization and this triggers action potential firing (Bigiani et al., 2003). This process can happen by two different channels. The first is the epithelial Na channel, ENAC (Bigiani et al., 2003). This pathway is Na selective. It is also inhibited by amiloride (a potassium sparing diuretic): the ENAC channel is amiloride -sensitive (AS) (Bigiani et al., 2003). Salty taste will be reduced by the presence of amiloride if a salt goes through this channel. The other pathway is amiloride- insensitive (AI). This occurs through permeation of a tight junction by a cation into the taste receptor cell through a Na transport system (Mierson et al., 1996). The AI pathway is not specific to sodium. The salty taste will

vary with the ion; the more similar to NaCl in terms of size and weight, the saltier it is perceived to be (Ye et al., 1991). A study done on rats showed that Na salts will go through

different channels depending on the size of the anion they are connected to (Ossebaard and Smith, 1995). Na with large salts will activate only the apical channels, the amilo-sensitive channels, while salts with small anions like NaCl activate both channels, the amilo-senstitive and insensitive (Ossebaord and Smith, 1995). K, Ca, Mg, and other salts are part of the amiloride insensitive channel. Because of the size of amiloride it is not able to fit through tight junctions in the membrane (Ye et al., 1993), and therefore these minerals are not affected by it. NaCl and other halide salts like KCl are able to fit through smaller junctions and will not be affected by amiloride where another larger molecular weight salt such as Na-gluconate would would not fit through these junctions and would be inhibited by amiloride (Ossebaard and Smith, 1995). Salts that are able to amiloride insensitive would act more similar to NaCl and therefore should be able to be perceived as being salty in taste.

Salt Replacers

Many halide salts will give a salty taste. A list of some halide salts and their taste properties are shown in Table 1.2. Unlike NaCl, however, these salts do not have a purely salty taste (Schiffman and Erickson, 1970). Most halide salts will have at least two different tastants: most are salty and bitter or salty and sour. The dominant taste will change depending on the concentration. For example, NaCl at low concentrations will have a bitter and sour taste, but at high concentrations NaCl has a purely salty taste (Murphy et al., 1981). Other salts are not as drastic as NaCl and will not have a pure taste but as the concentrations change different tastants will be the major contributing taste. The anion and the cation in the salt contribute to

the taste: the heavier the anion/cation, the more bitter tasting the salt will be and the lighter the anion/cation, the saltier the taste will be (Murphy et al., 1981). The major cations that have salty taste responses are sodium, lithium, potassium, calcium, magnesium, rubidium, and caesium. The major anions used are chloride, iodine, and bromide.

The most popular, major halide that has a salty taste and can be used as a salt replacer is Potassium (K). Potassium, like Na, is an electrolyte and is involved in membrane potential and electrical excitation of nerve and muscle cells (Vaskonen, 2003). It has been shown to decrease blood pressure. In one study, an increase of K by 1.9g/day decreased blood pressure by 4.5 mmHg systolic blood pressure and by 2.5 mmHg for diastolic blood pressure (Whelton et al., 1997). According to the Dietary Guidelines for Americans, the adequate intake (AI), the amount that is needed daily for healthy adults, for K is 4700 mg/day (USDA, 2010). The average intake in the United States is below this level. The most popular salt substitute is potassium chloride (KCl), although, other ionic potassium salts can have a salty taste. Different anions with K will affect whether the salt is perceived as salty or as more bitter or sour (Murphy et al., 1981). The salty taste of K salts can be seen in Figure 1.2. KCl had a bitter taste below 0.108M. Above this concentration it becomes salty. However, potassium bromate (KBr) is both equally salty and bitter at concentration below 0.108M (Murphy et al., 1981). K had overall lower salty taste than sodium, but it follows the trend where having an anion that is an organic acid or other halide salt decreases salty taste more than if Cl was the anion (Schulkin, 1982).

KCl has a bitter/metallic aftertaste (Murphy et al., 1981; Sinopoli and Lawless, 2012). Sinopoli and Lawless (2012) found that KCl increased in saltiness perception with concentration but increases in other tastes/flavors were also found. At the highest concentration of KCl used (0.6%), the tastes/flavors consumers detected in KCl were bitter,chemical, metallic, plastic, salty, drying, sour, moldy, and no taste. Lower concentrations had similar taste/flavors just at lower frequencies. KCl is usually used in conjunction with NaCl to help reduce the bitter taste. NaCl is known to reduce bitterness in many bitter compounds (Keast and Breslin, 2002; Breslin and Beauchamp, 1995; Kroeze and Bartoshuk, 1985; Schifferstein, 1994; Sonpoli and Lawless, 2012). A study conducted by Breslin and Beauchamp (1995) looked at the different interactions between NaCl and different bitter compounds. NaCl (concentration: 0, 0.05, 0.10, and 0.20M) was added to KCl in the same concentrations. All concentrations of NaCl inhibited the bitterness of KCl. At the highest concentration of NaCl, the bitterness of KCl was reduced by 78% (Breslin and Beauchamp, 1995). A solution of KCl and NaCl was also saltier in taste than just that of NaCl alone. KCl can provide a 15-20% reduction in salt while still providing acceptable taste in many foods (Batenburg and Velden, 2011). Other studies showed a higher percentage of substitution with other K salts or a mixture of K salts were acceptable in bitter taste. A British study reduced sodium content within bread by using different potassium salts: Potassium citrate(K-cit) and Potassium Bicarbonate (K-bic). The potassium salts that produced the highest overall liking in the breads were substituting 30% NaCl with K-bic or substituting 30% NaCl with a 1:1 mixture of k-cit and KCl (Braschi et al., 2009). However,

the breads that had the best flavor contained 30% substitution of NaCl with KCl and K Bic (Braschi et al., 2009).

Potassium is already used as a salt replacer in some commercial products. Morton salt has both a "lite" salt and a full salt replacer. These products both replace NaCl with KCl (Morton Salt, 2011). The lite version is 50% less NaCl. According to the label, in each half teaspoon it contains 550 mg of sodium and 733 mg of potassium while normal salt has 1200 mg per half teaspoon (Morton Salt, 2011). A study done on Morton "lite" salt compared it to regular salt in cooking (Kincaid et al., 1976). The taste of the "lite" salt when it was used to cook rice was identical to consumers as regular salt (Kincaid et al., 1976).

Other halide, non-sodium, salts that have a salty taste and are linked to lowering blood pressure are calcium (Ca) and magnesium (Mg) salts (Vaskonen, 2003). Calcium has many health benefits and is recommended to the public to lower many different chronic diseases (Vaskonen, 2003). The RI for calcium is 800mg/day but up to 2000mg/day is considered safe (Vaskonen, 2003). According to Vaskonen (2003), if current calcium intakes were increased by 100mg it could decrease blood pressure by 0.39mmHg systolic and by 0.35mmHg diastolic (Vaskonen, 2003).

Calcium salts are mostly bitter with low salty taste responses that increase slightly over time. Calcium lactate was generally less intense then calcium chloride (Lawless et al., 2003; Tordoff, 1996). At suprathreshold concentrations, calcium salts were considered unpleasant by subjects (Tordoff, 1996). Tordoff (1996) tested many different calcium salts to determine the taste and confirmed that calcium chloride was the best for salty taste substitution.

Calcium lactate also had some salty taste. At low concentrations (1mM), calcium chloride was considered to be 35% bitter, 32% sour, 24% sweet and 4% salty; however, at higher concentrations (100mM) it was 44% bitter, 20% sour, 1% sweet, and 35% salty (Tordoff, 1996). In the higher concentrations of both calcium lactate and calcium chloride, as the concentration went up and the intensity of non salty tastes such as bitter or sour increased, the overall liking of the salts went down (Tordoff, 1996). Although the saltiness increased,the higher perception of bitter taste drove the liking scores down. Calcium salts were more bitter than sodium salts across all anion combinations.

Magnesium salts also are thought to lower blood pressure (Vasoken, 2003). The RDA for Mg is 400 mg/day (Vaskonen, 2003). Like Ca, Mg is also salty and bitter. However, Mg salty taste responses stayed consistently low with concentration (Lawless et al., 2003): saltiness did not increase as the concentration increased like most of the other salts. Because of the bitter taste of these salts, like K, if used as salt replacements they would have to be used with another compound to suppress the bitter taste.

One study tested each of the above mentioned halide salts to try and reduce the sodium levels in cookies (Bala et al., 2004). They replaced NaCl with KCl, $MgCl_2$, $CaCl_2$, $MgSO_4$, and Na_2SO_4. They also replaced the sodium in baking soda with K and a combination of $NaHCO_3$ and NH_4HCO_3 (50:50). They found that the cookies made with the substituted salts were all acceptable. The order of acceptability from highest to lowest was KCl, Na_2SO_4, $MgSO_4$ $MgCl_2$ and $CaCl_2$ (Bala et al., 2004). The taste ratings were established in the flours first and it was discovered that CaCl2 actually could not be baked with. The baking sodium

replacement that was most favored was KCl. When NaCl was replaced with these substitutes it reduced the sodium content by almost one third (Bala et al., 2004).

Lithium (Li) salts have similar salty taste responses as Na (Murphy et al., 1981). LiBr and LiCl have high salty taste responses with greater concentrations. A study done on sodium deprived rats showed that rats will ingest Li salts because of the salty taste that it has (Schulkin et al., 1982). Li salts also follow the same pattern as sodium as far as the anion it is attached to and salty taste (Schulkin, 1982). Li, like Na, is part of the amiloride sensitive channel: its salty taste would be reduced by the presence of amiloride. As a pure salt replacer, Li does not work because Li is toxic at levels above 1.5 mEq/L (Timmer and Sands, 1999)Other salts like caesium and rubidium have a mild salty taste response (Murphy et al.,1981). These salts are mostly characterized by having bitter or salty taste (Murphy et al., 1981). Figure 1.3 shows different salts and their saltiness.

Flavor Enhancers

Several studies have suggested that organic acids, amino acids, and nucleotides can add flavor to foods (Schiffman et al., 1980; Salles et al., 2002; Kang et al., 2007). Amino acids are present in many foods and are important stimuli for tastes: they provide complex tastes (Schiffman et al., 1980). Glycine, L-proline, L-serine provided sweet tastes while basic amino acids had a salty taste (Schiffman et al., 1980). 5'-nucleotides also add an agreeable taste to foods (Sakaguclii, 1963).

Although these compounds (amino acids, nucleotides, and organic acids) may contribute a unique taste themselves, most work by enhancing the salty taste or suppressing a bitter taste. These compounds would be used as a flavor enhancer and not a salt replacer. A salt replacer

would be a compound like the halides discussed earlier that are added either to completely remove NaCl or as a partial replacement of NaCl. A flavor enhancer works not by replacing the NaCl present but by maximizing a flavor that is already present. In a study done by Breslin (1999), he defined enhancement as "a linear (additive) process that bears direct analogy with subthreshold hyper additive mixing." In simple terms, a flavor enhancer is a substance that heightens a taste that is already present in a food. According to Katsumata et al. (2008), "an ideal enhancer is: tasteless, odorless, non-pungent, stable at high temperatures, effective at low concentrations, effective in modulating salt receptors, reversible, and additive."

Several studies have used organic acids such as acetic, citric, malic, and tartaric to enhance the salty taste of NaCl (Fabien and Blum, 1942; Kamen et al., 1961; Pangborn, 1959; Pangborn, 1963). Helleman (1992) showed that at low concentrations of NaCl, lactic and acetic acids were able to enhance saltiness. This experiment was conducted in water and in a food matrix, bread. In water, the acids increased the salty taste of sodium with increasing amount of acids. However, at high concentrations of NaCl, salty taste actually decreased with increasing concentration of the acids. In bread this effect was not observed. At all concentrations of NaCl in bread, the acids enhanced the taste of NaCl.

Kang et al. (2007) also found that lactic acid increased perceived salty taste in ramen style soup. At a concentration of 0.0066%, lactic acid increased saltiness and beefiness in soup. However, at a concentration of only 0.0039%, lactic acid did not enhance salty taste. This study along with the Hellemann study, suggests that organic acids are able to enhance salty

taste at the ideal concentrations. The Hellemann (1992) study demonstrated that if the salt concentration goes above a certain percentage, the enhancement effect is lost. However, if the acid concentration is not high enough it does not provide any enhancing behavior to the salt that is present.

One study compared three organic acid sodium salts and the salty taste response they elicited in blowflies (Murata et al., 2001). The organic acids used were acetic, succinic, and citric. When tested by themselves the organic acids had no salt response, but when added to sodium they all yielded salt responses higher than the salt alone. The organic acid which had the highest perceived salty taste intensity in the flies was acetic acid; it was followed by succinic and then citric acid (Murata et al., 2001). The same experiment was done to see if the same organic acids enhanced the saltiness of KCl. The same organic acids enhanced the salty taste of KCl; however, a higher concentration of the acid was required in the KCl solutions than in NaCl to enhance salty taste. In this same study, organic acid sodium salts of the organic acids previously added to NaCl solutions were tested for salty taste intensity compared to NaCl salts. Sodium acetate had a stronger salty taste response than that of NaCl. The two other sodium salts, sodium citrate and sodium succinate, did not have as strong of a salty taste response as that of NaCl. Acetate raises the sensitivity of the salt receptor cells to sodium ions (Murata et al., 2001). A solution that has an acetate salt in it will taste saltier than pure NaCl at the same concentration.

US Patent 4,243,691 used a combination of non-sodium salts and sodium enhancers to generate a salt substitution product (Mohlenkamp and Hiler, 1981). The product was 10-

40% by weight flavor enhancer amino acids, 10-40% K phosphate, KCL, and 5'-nucleotides. The combination of ingredients enhanced the salty taste. There was no bitter taste from the K because the nucleotides suppressed the bitter taste. A lower amount of K salts were needed to create a salty taste than would be needed if the nucleotides were not present.

Another patent used lactose or dextrose to mask the bitter taste of a KCl and ammonium chloride mixture (Eisenstadt, 1975). Small amounts of the sugar and potassium hydrogen tartrate (cream of tartar), in very small amounts, were needed to suppress the bitter taste that was associated with both of these salts. Although lactose or dextrose alone can inhibit bitter taste, when mixed with cream of tartar, bitter taste was further reduced (Patent 3860732).

Umami and Salty taste

Many compounds that contribute to umami taste are salt enhancers. Umami is the fifth basic taste. It is elicited by glutamates (Fuke and Ueda, 1996). This includes monosodium glutamate (MSG), guanosine monophosphate (GMP), adenosine monophosphate (AMP), and inosine monophosphate (IMP). Peptides are also able to produce an umami taste (Lioe et al., 2006; Schlichtherle-Cerny and Amada, 2002). Examples of synthesized peptides that have an umami taste are Gly-Asp, Ala-Glu, Gly-Asp-Gly, Val-Asp-Val, Asp-Leu and Val-Glu-Leu (Ohyama et al., 1988). These produce a lingering umami taste. However, umami taste is most closely related to monosodium glutamate. In the early 1900s, Ikeda (1912) discovered metallic salts in brown kelp. He recognized that MSG gave the food a desirable flavor (Ikeda, 1912). Umami has a savory, meaty taste to it and is sometimes known as "the taste of proteins" (de Araujo et al., 2003). The umami taste is perceived by receptors on the tongue that respond to MSG (Bellisle, 1999). This taste receptor is a G coupled protein and

functions similar to the taste receptor cells that generate a sweet or bitter taste (Fuke and Udea, 1996). MSG is also known to have a synergistic effect when combined with some ribonucleotides (Hayashi et al., 1994).

MSG and other nucleotide flavor enhancers, AMP, IMP, etc. belong to one of two classes structurally. The two classes were L-amino acids containing 5 carbon atoms or purine ribonucleoside 5'-monophosphates having a 6-oxy group: MSG belongs to the first group and AMP, IMP, and GMP belong to the second class (Maga and Yamaguchi, 2009). Depending on the structure, nucleotides change their function and their ability to enhance savory flavors and salty taste. Figure 4 shows the different structures of the different mononucleotides.

A study tested the different flavor enhancing compounds in sausages (Ruusunen et al., 2001). In this study, they compared MSG to Ribotide. Ribotide is a commercial flavor enhancer which is a combination of MSG, IMP, and GMP. The sausages that were made with MSG alone with no other added mononucleotides were perceived as having the highest amount of salty taste (Ruusunen et al., 2001). However, after 27 days of storage the sausages with MSG or Ribotide were not different. This result suggests that other mononucleotides have the ability to enhance salty taste perception.

MSG was shown to have a similar taste with that of NaCl. In a study on rats, rats were conditioned to be adverse to the taste of MSG. When the rats were then presented with NaCl, they avoided consumption (Bellisle, 1999). Yamaguchi and Takanashi (1984) conducted a study on clear soups with MSG and its effect on salty taste. They found that MSG enhanced

salty perception: less salt was needed when more MSG was added or vice versa (Yamaguchi and Takanashi, 1984). The optimal combination was 0.38% MSG to 0.81% salt.

MSG and calcium diglutamate (CDG) were studied for their effect on lower sodium soups (Ball et al., 2002). MSG had been shown to enhance salty taste in other studies (Okiyama and Beauchamp, 1998; Roininen et al.,1996; Yamaguchi and Takahashi, 1984) but because it has a Na ion connected it also adds a small amount of sodium to the food. Because of this, CDG was tested to see if the effects were the same. CDG would also add the health benefits of the calcium ion. Researchers found that when NaCl levels were reduced to 85mM and a glutamate was added the soups had equal or higher salty taste intensity (Ball et al., 2002). They also found that there was no difference between adding MSG or CDG.

Flavor suppressants

Basic taste compounds in the presence of other basic tastes can alter the way that a substance is perceived; if one taste lowers the intensity of another it is called suppression. Sweet taste is the most common suppressant of other tastes, and provides the strongest taste (Green et al., 2010). Sucrose is known to suppress in binary mixtures the saltiness of NaCl, the sourness of acids, and bitterness in both caffeine and quinine (Pangborn, 1959; Hopkins, 1953; Breslin 1996). In a binary mixture, sucrose is able to suppress the salty taste intensity of NaCl by 82.6% (Green et al., 2010).

Bitter taste is also known to be suppressed by sodium salts (Breslin and Beauchamp, 1995; Keast and Breslin, 2002). Keast et al. (2006) evaluated the effect of Na salts on different bitter compounds using the gLMS scale. The gLMS scale is a logarithmic scale but there are no numbers on the scale. The scale is identified with words from weak to the strongest

sensation ever experienced. Solutions were made with varying bitter compounds and Na salts. All the different Na salts decreased the expression of bitter taste in most of the bitter compounds used except for tetralone, an iso-alpha- acid which is the primary bitter compound in beer (Keast et al., 2004). In a study conducted on suppression of bitter taste of whey protein hydrolysate, sodium chloride and some salty taste enhancers suppressed bitter taste in beverages (Leksrisompong et al., 2012). Whey protein hydrolysates (WPH) at two different levels of hydrolysis were tested (5% and 32% degree of hydrolysis) with potential bitter taste by descriptive analysis. The substances that reduced bitterness were then evaluated in beverage formulations. Sodium chloride suppressed bitterness of the WPH beverage made from WPH with a higher degree of hydrolysis. Other substances such as

monosodium glutmate and AMP, which are salty taste enhancers, also reduced bitter taste in WPH beverages (Leksrisompong et al., 2012).

The findings for suppression are dependent upon concentration, technique used, flow directly over tongue or swallowing motions, and if the panel is trained or untrained so it is difficult to say that one tastant will always suppress or enhancer another (Breslin, 1996). Most likely, at one concentration it will enhance/suppress and at another concentration is may have the opposite effect or no effect at all. A general trend for the basic tastes effect on one another is presented in Table 1.4.

Dairy studies and sodium reduction

Many studies have been conducted to reduce the sodium content in various foods. A large number of these studies were done in dairy products, especially cheese. Reducing the sodium content in cheeses would contribute to the overall intake of sodium by consumers (Agarwal

et al., 2011). In this study, they reported various Na levels that were present in a variety of cheeses. In Cheddar cheese, the average sodium content was 615 mg/100g (Agarwal et al., 2011). Mozzarella had an average Na content of 666 mg/100g (Agarwal et al., 2011), and processed cheeses had the highest Na concentration with an average of 1,242 mg/100g (Agarwal et al., 2011). Since cheese is a significant source of sodium in the diet (Albernethy, 1979; Anon, 1980), reducing the sodium levels in these cheeses could reduce the population sodium intake.

Because sodium plays a large part in natural cheese ripening many studies have been done to develop low sodium products (Katsiari et al., 1997). When sodium is taken out of natural cheese, and no replacement is used, problems develop with cheese flavor and texture

(Anonymous, 1992; Johnson et al., 2009). KCl was used to replace some of the sodium to reduce these problems (Reddy and Marth, 1991). Partial Na reduction with KCl was done in Minas fresh cheese (Gomes et al., 2011). In the study, four different cheeses had varying levels of KCl replacement for NaCl: 0,25,50,75 %(wt/wt) (Gomes et al., 2011). The control product, which had no Na replacer, had the highest liking scores, the 25 %(wt/wt) replacement was acceptable; it had scores that were not significantly different from the control (Gomes et al., 2011). The 50% and 75% substituted cheeses were not liked by consumers. The bitter taste that is associated with K was perceived in these cheeses.

A similar project was done in Feta cheese by Katsiari et al. (1997). Feta cheeses with a 3:1 or 1:1(w/w) mixture of NaCl and KCl were acceptable low sodium products. This study evaluated not only flavor but texture. Both of the mixtures were not significantly different

from the control cheeses in sensory evaluation (Katsiari et al., 1997). Although in the 1:1 mixture a slight metallic taste was perceived by some of the panelists; this mixture, however, still remained acceptable. The 3:1 mixture did not have a bitter taste detected because of the masking influence of NaCl (Katsiari et al., 1997; Charteris and Keogh, 1991). Texture evaluation was also done on the reduced sodium Feta cheeses. The cheeses that were made with the mixture of NaCl:KCl were softer than the control cheese (Katsiari et al., 1997).

A study on a variety of dairy products was conducted to determine if sodium levels could be reduced and consumer acceptance maintained (Drake et al., 2011). The dairy products that were used were milk based soups, cottage cheese, and a Cheddar cheese sauce. The soups and the cheese sauce had sodium reductions from 5%-35% done in 5% increments. The cottage cheese started at 3% reduction and went up to 21% reduction in increments of 3%.

Sodium reductions in the soups and the sauces were less detectable than in the cottage cheese (Drake et al., 2011). These authors reported that consumers were able to detect a difference in NaCl in the varying products at different percentages. The differences detected were: 8% reduction in cottage cheese, 15% reduction for the milk based soups, and 14% reduction in the cheese sauce.

Another unpublished study, used whey permeates as a Na substitute in cream based soups (Dixon, 2008 MS thesis, unpublished). Permeate was added to two different soup bases: canned and fresh. The permeate version of the canned soup had 19% of the Na of the full sodium version and the fresh permeate soup had 11% of the Na in a full Na soup (Dixon, 2008 MS thesis, unpublished). Each of these were compared to soups with 0, 50, 100% of the

standard content of Na. The permeate soup for the canned version ranked slightly saltier than 50% of the full Na. The fresh permeate soup ranked slightly lower than the 50% standard soup sample (Dixon, 2008 MS thesis, unpublished).

Permeate

Many foods naturally have flavor enhancers in them; one of these foods is cheese (Maga and Yamaguchi, 2009). These enhancers include nucleotides, organic acids, and minerals naturally found in cheeses (Maga and Yamaguchi, 2009). However, only about 10% of these remain in the finished cheese product. The rest is removed in the whey after curd formation. The minerals do not remain in the whey. During processing of whey into value added ingredient, minerals, organic acids, and nitrogenous compounds are filtered and concentrated by ultrafiltration and end up in the permeate. According to the United States Dairy Export Council, whey permeate is "a source of dairy solids obtained from proteins and some minerals in whey" (USDEC, 2011). Whey is a greenish- yellowish liquid that is leftover from the manufacture of cheese. Whey protein is concentrated and used as a protein source in many products. In order to concentrate the protein, lactose and other minerals are removed; this is done through ultrafiltration. The lactose and minerals are the compounds that end up in permeate. Permeate will account for approximately 90% of the whey volume (Chandan et al., 1982). Figure 1.5 shows the overall process for the production of whey permeate.

There are many types of permeate: whey, milk, and reduced lactose permeate. Milk permeate is the byproduct of when milk is concentrated to make milk protein concentrate/isolate. Milk is ultrafiltered to concentrate caseins and butterfat; the liquid that is

leftover is milk permeate (Animal Feed Sources Information, 2011). Milk permeate is mostly lactose with some soluble proteins and minerals (El-Khair, 2009).

Reduced lactose permeate has about 20-55% of the lactose crystallized removed. The production of reduced lactose permeate is outlined in Figure 1.7. Permeate is first concentrated in the evaporator. After concentration it is put into a crystallization tank where starter lactose seed crystals are added and lactose (20-55%) is crystallized from the permeate. Once the crystals become a large enough size they are centrifuged from the rest of the solution. The liquid remaining is reduced lactose permeate.All the varying types of permeate can be found in both liquid and solid form. The liquid permeate is the liquid left after the whey/milk has been ultrafiltrated. The solid is the spray dried version. This makes the solid version slightly more expensive but easier to work with (USDEC, 2011). The production of permeate has increased from 2005-2009 by 17% each year (Gerdes, 2011). The composition of permeate is variable (Table 1.5, 1.6).Reduced lactose permeate is higher in mineral concentration because other components are concentrated before the lactose is removed from the permeate. Permeate can promote browning, enhance flavor, improve moisture retention, and reduce sodium (USDEA, 2011). It is currently found in infant formula, instant drinks, bakery products, confectionary bars, instant soups, milk drinks, sweetened condensed milk, ice cream, and dessert products (USDEC, 2011).

While very few studies have been done on permeate, many of them have worked with hydrolyzing the lactose to replace sucrose in products. Hydrolyzed whey permeate replaced about 50% of sucrose in peaches without any detectable differences in quality of the peaches

(Tweedie and MacBean, 1978). Another project used hydrolyzed and hydrolyzed-isomerized whey permeate in ice cream (Arndt and Wehling, 1989). Whey permeate was hydrolyzed and made into syrups of 25% and 50% replacement of sucrose. The ice cream were tested for overall acceptability, appearance, body, texture, and sweetness. The ice creams made with 25% and 50% syrups were acceptable as a substitute across all attributes. The hydrolyzed-isomerized 50% resulted in the sweetest ice cream.

More recently, permeate was studied as a component in a sports beverage (Beucler et al., 2005). The beverages were created using whey permeate and hydrolyzed whey permeate. Varying percentages of deionized water and permeate were used. The percentages used were as follows: 0%, 25%, 50%, 75% and 100%. The hydrolyzed permeate had higher sweeter taste than the non hydrolyzed permeate. The beverages were salty but comparable in salty taste to a commercial sports beverage. Both permeates were acceptable at 25% and 50% of the beverage volume. Low levels of permeate could be added to a sports beverage. There are also some studies that suggest that permeate can potentially be used as a salt replacer (Dixon, 2008 MS thesis unpublished). Although it has had some success as a salt replacer in products, it is not known what compounds in permeate contribute to salt replacement. The objective of this study was to determine the sensory properties and composition of whey permeates, and to demonstrate if components other than Na are responsible for the salty taste.

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TABLES

Table 1.1 Daily Nutrient Goals Used in DASH Studies. Based off of 2100 calorie diet. (US Department of Health and Human Services, 2006)

Total fat	*27% of calories*
Saturated fat	*6% of calories*
Protein	*18% of calories*
Carbohydrate	*55% of calories*
Cholesterol	*150 mg*
Sodium	*2300 mg*
Potassium	*4700 mg*
Calcium	*1250 mg*
Magensium	*500 mg*
Fiber	*3 g*

Table 1.2 Approximate taste of some halide salts (Schiffman and Erickson, 1970)

Stimulus	*Approximate taste*
NaCl	*Pure Salty*
LiCl	*Salty*
NH_4Cl	*Salty-sour or salty-bitter*
KCl	*Salty-bitter*
$MgCl_2$	*Salty-bitter*
$CaCl_2$	*Salty-bitter*
NaAc	*Salty or salty- sour*

Table 1.3 Summary of sodium replacers and salty taste enhancers

Compound	Taste	Salt replacer	Flavor enhancer	Used in	Studies
Organic acids -Acetic - Lactic - Citric	-Bitter -Sour -Salty	Yes Organic salts	Yes	-Can be used to suppress a tastant -Providesalty taste on own	-Soup as enhancers (Kang et al., 2007)
Potassium - KCl - KBr - K-cit - K-bic	-Bitter -Metallic -Salty	Yes	No	-Sodium replacers (Morton Lite salt) - Food products- partial replacement	-Breads (Braschi et al., 2009) -Non sodium salts (Braschi et al., 2009) -Cookies (Bala et al., 2004) -Meats (Ruusunen and Puolanne, 2004) -Processed Foods
Calcium - Calcium Lactate - CaCl2	-Bitter - Salty	Yes	No	-Food products partial replacement	-Cookies (Bala et al., 2004) -Meats (Ruusunen and Puolanne, 2004) -Processed foods
Magnesium - MgCl2 -MgSO4	-Bitter -Salty	Yes	No	-Food product partial sodium replacement (30% of NaCl)	- Processed foods -Meats -Cookies (Bala et al., 2004)
Lithium	-Salty	N o	No	-Can be toxic (<1.5mEq/L) at certain amounts added to foods	

Table 1.4 Supression/enhancement of the basic tastes

Taste	Suppressed by	Enhanced by
Salty	Sweet (Pangborn, 1959)	Sour (Breslin,1996), Umami (Ball et al., 2002)
Sweet	Sour (Pangborn, 1961), Bitter (Prescott,2001)	NaCl (Pangborn, 1959)
Sour	Sweet, Salty, Bitter (Pangborn, 1959)	_____
Bitter	Salty, Sweet, Umami (Keast et al., 2004)	_____
Umami	Sweet (Sako et al., 2003)	_____

Table 1.5 Composition of whey permeate (USDEC, 2011)

Compound	Percentage of Total Solids
Lactose	65-85%
Ash/minerals(Potassium, sodium, Calcium, Magnesium)	8-20%
Protein	3-8%
Fat	Max 1.5%

Table 1.6 Mineral composition of whey permeate and reduced lactose whey permeate (USDEC, 2011)

Mineral	Permeate (% w/w)	Reduced lactose permeate (% w/w)
Sodium	0.83%	2.00%
Calcium	0.44%	3.76%
Potassium	2.47%	6.29%
Magnesium	0.11%	0.24%

FIGURES

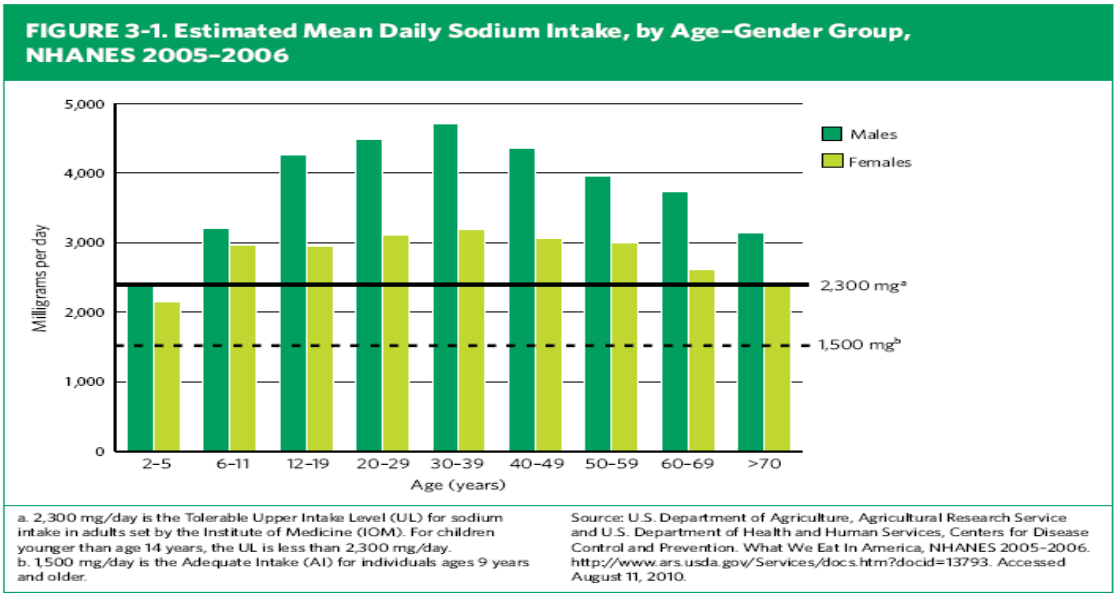

Figure 1.1 Estimated Mean Daily Sodium Intake

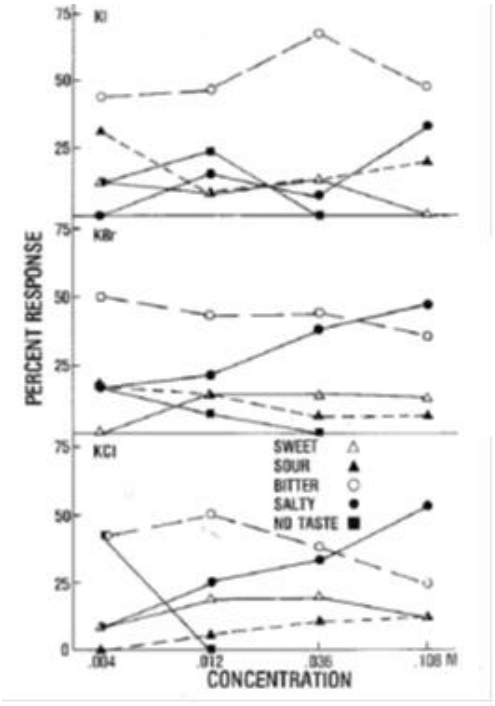

Figure 1. 2 Potassium salts and salty taste response. Percent response gives the number of responses for each taste given by tasters not the intensity of the taste.

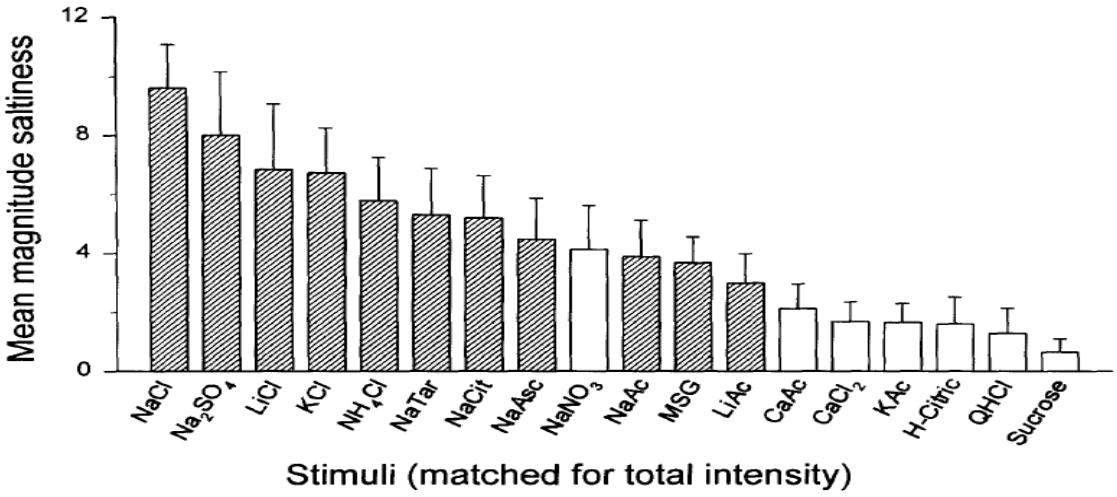

Figure 1.3 Mean magnitudes of saltiness for 18 stimuli at best concentration

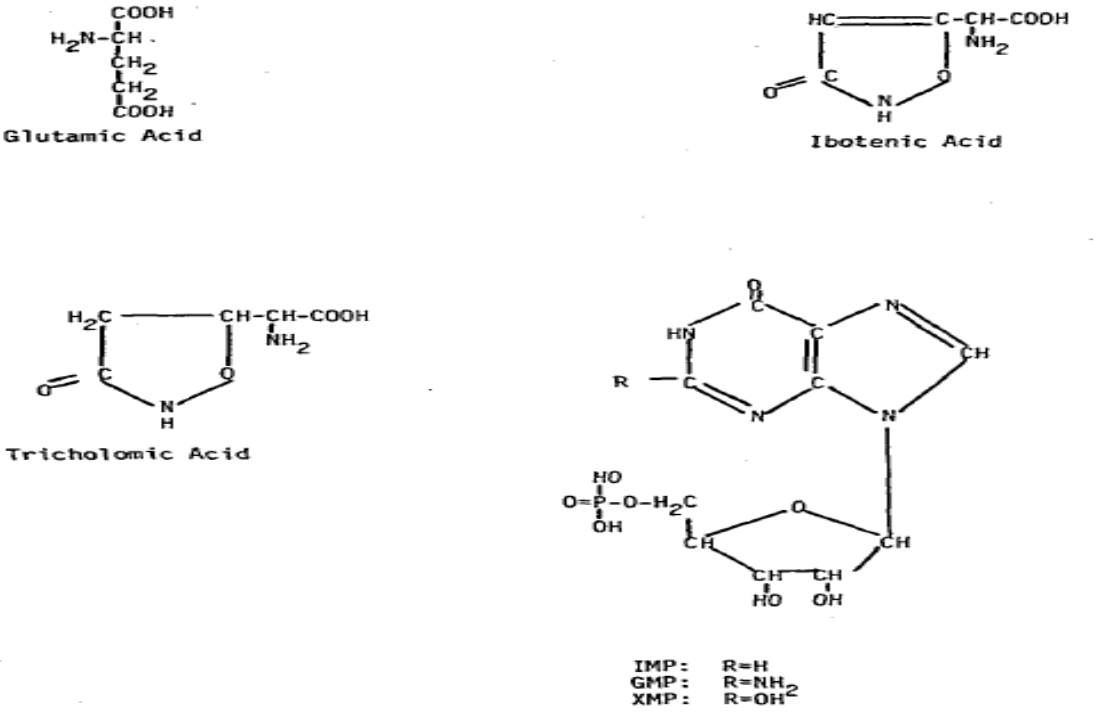

Figure 1.4 Example structures of nucleotides that enhance savory flavors and salty taste. (Maga and Yamaguchi, 2009)

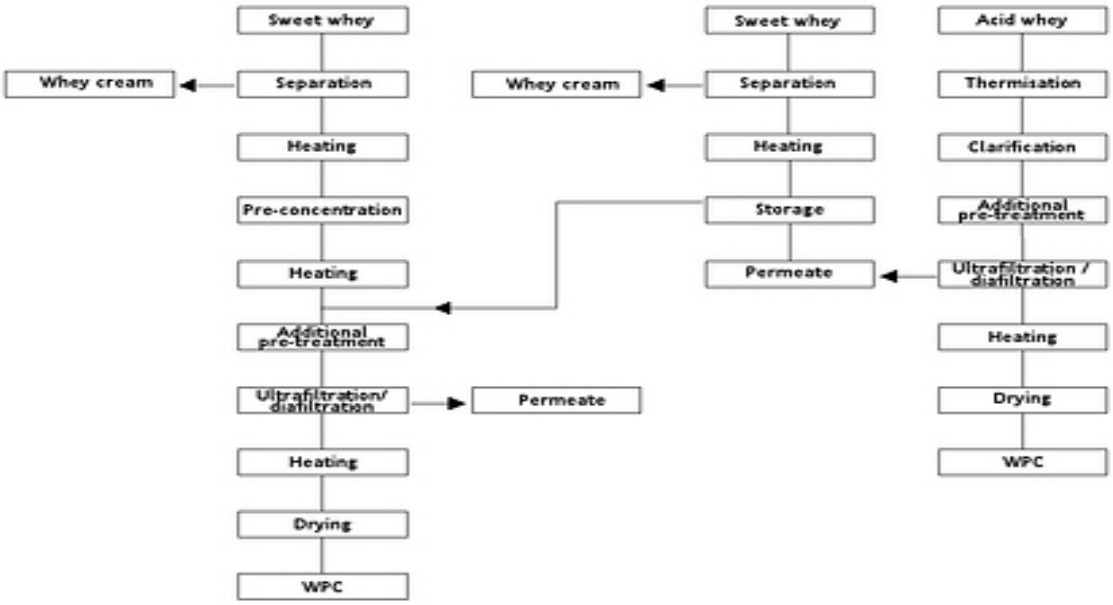

Figure 1.5 Flow chart for whey permeate production (USDEC, 2004)

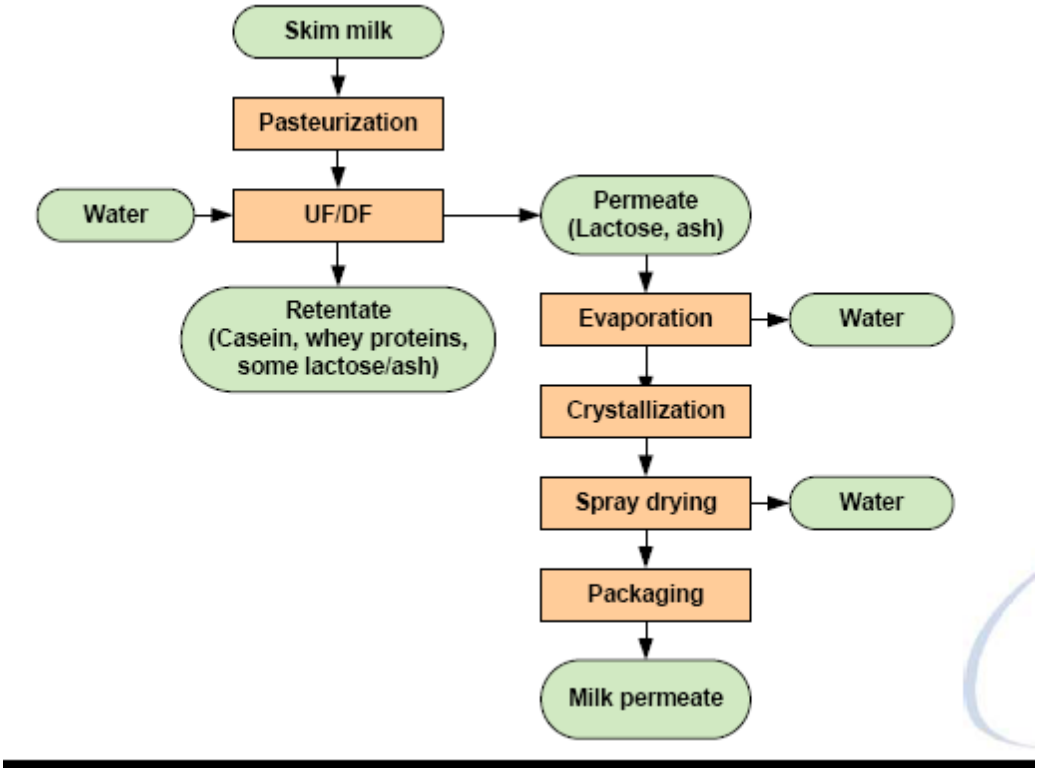

Figure 1.6 Process for milk permeate (Wisconsin Center for Dairy Research, 2010)

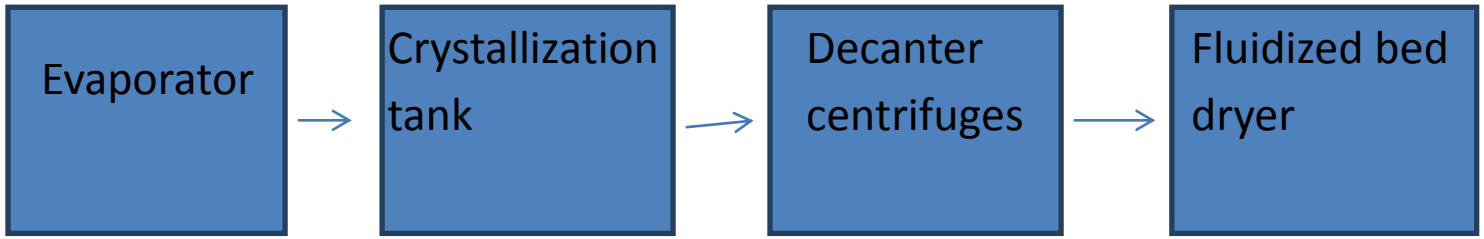

Figure 1.7 Process for reduced lactose permeate

CHAPTER 2

The Role of Sodium in the Salty Taste of Permeate

The role of sodium in the salty taste of permeate

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Abstract

One of the major contributing factors to hypertension in the U.S. is from the amount of sodium in the American diet. Many food companies are trying to limit the amount of sodium in their products. Permeate, the liquid remaining after whey or milk is ultrafiltered, has been suggested as a salt substitute. It has not been established what part of permeates composition allows for its use as a sodium replacer or if permeates exhibit salty taste. The objective of this study was to determine the sensory properties of permeates and their composition to determine if elements other than sodium (Na) contribute to the salty taste found in permeate. Eighteen whey (n=14) and reduced lactose (n=4) permeates were obtained in duplicate from commercial facilities. Proximate analyses, specific mineral content, and non protein nitrogen were determined. Organic acids and nucleotides were extracted followed by high performance liquid chromatography (HPLC). Aromatic volatiles were evaluated by gas chromatography mass spectrometry (GCMS). Descriptive analysis of permeates and model solutions was conducted using a trained sensory panel. Correlation analysis was ran to determine which compounds were likely associated with salty taste. Whey permeates were characterized by cooked/milky and brothy flavors, sweet taste and low salty taste. Permeates with lactose (DLC) removed were distinctly salty. The organic acids with the highest concentration in permeates were lactic and citric acid. Volatiles included aldehydes, sulfur containing compounds, and diacteyl. Sensory tests with sodium chloride solutions confirmed salty taste of DLC was not solely due to sodium present. Models were created, based off

correlation analysis, with KCL, lactic acid, citric acid, hippuric acid, uric acid, orotic acid, and urea. KCl, lactic, and orotic acid were found to enhance the salty taste of permeates.

Introduction

Currently Americans consume too much salt. The average intake of sodium chloride in the U.S. is 3000 mg per day while the recommended intake is 1500-2400 mg a day (USDA, 2010). Most of this salt comes from processed foods and is not from addition of salt to food (Anderson et al., 2009). High sodium intake is linked to many adverse health conditions including hypertension and cardiovascular disease. According to the United States Department of Health and Human Services (2006), 65 million Americans suffer from hypertension.

Many studies have shown the correlation between sodium and high blood pressure; however, people are still hesitant about low sodium diets (FDA, 2008; Kim et al., 2012). Fifty percent of consumers would like to decrease their sodium but don't want to lose the flavor of their foods (NMI, 2008). Kim et al. (2012) recently demonstrated that consumers were aware of a link between certain medical conditions like hypertension and high sodium consumption. Even though consumers were aware of this relationship, salt was considered a "must have" attribute by consumers in food. Consumers also preferred a "reduced" sodium version of the food product compared to a sodium "free" product.

The food industry is currently trying to reduce the amount of sodium in food products (Berry, 2010). Sodium reduction can be achieved by changes in formulation as well as use of other salt replacers to help provide the water activity and flavor properties contributed by salt. Salt replacers are often other halide salts, such as potassium chloride (KCl). These salts usually have another basic taste besides salt in them. KCl is perceived as salty, bitter, and metallic

(Murphy et al., 1981; Sinopoli and Lawless, 2012). KCl is often used in conjunction with NaCl; as NaCl:KCl blends reduce the bitter taste of the KCl (Breslin and Beauchamp, 1995).

Other compounds do not contribute taste to a product but will enhance a taste that is already found in the product; these are known as flavor enhancers. The most common flavor enhancer is monosodium glutamate. Organic acids, nucleotides, and non protein nitrogen compounds are also used to enhance flavor (Schiffman et al., 1981; Salles et al., 2002; Kang et al., 2007). When organic acids were added to Na and K they increased the salty taste response of blowflies (Murata et al., 2001). The acid that elicited the highest increase in salty taste was citric acid. Lactic acid was also used successfully to increase salty taste in Raman style soups (Kang et al., 2007). Nucleotides also have an effect on salty taste. MSG has been shown to enhance salty taste in other studies (Yamaguchi and Takahashi, 1984; Roininen et al.,1996; Okiyama and Beauchamp, 1998). Ball et al. (2002) reported that when NaCl levels were reduced to 85mM and glutamate was added, soups had equal or higher salty taste intensity to control NaCl levels (Ball et al., 2002). They also found that there was no difference between adding MSG or calcium diglutamate (CDG) in terms of enhancing salty taste in the soups (Ball et al., 2002).

Permeate, also known as dairy product solids, deproteinized whey, or modified whey, is the liquid that remains after ultrafiltration of whey or milk, and a byproduct of manufacture of whey and milk protein concentrate/isolate. Minerals and lactose removed during membrane fractionation are found in permeate. The approximate dry weight composition of permeate is 65-85% lactose, 8-20% ash/minerals, 3-8% protein, and less than 1.5% fat (USDEC, 2011).

Reduced lactose permeate has had a portion of the lactose crystallized out and has roughly three times the mineral content of regular permeate (USDEC, 2011). Permeate production has increased by about 17% per year from 2005-2009 (Gerdes, 2011). Whey protein ingredients are popular functional ingredients, but permeate is still mostly viewed as a by-product. Permeate currently has established applications in infant formulas and baked goods. Recent research proposed the use of permeate as a salt replacer in food products (Archwamety, 2012). Permeate contains sodium but it is not known if other components of permeate have salty taste enhancing properties. The objective of this study was to evaluate the composition of permeates and to determine the sensory properties of permeate and permeate components in order to determine if components in addition to Na were responsible for the salty taste.

Materials and Methods

Permeates

A total of 18 permeates (7 powders and 11 liquids) from 12 commercial suppliers were obtained in duplicate. These included whey permeates (n= 14) and reduced lactose whey permeates (n= 4). Upon receipt, liquid samples were stored at -20°C and powders were stored at 21°C until analysis (< 2 weeks).

Proximate Analysis

Total solids were determined using a vacuum oven (AOAC method 927.5). Fat was determined by mojonnier method (AOAC 989.05). Sodium, potassium, calcium,

magnesium, and ash content were determined by inductively coupled plasma spectrophotometry (ICP) by the North Carolina State Univ. Analytical Services Laboratory

(Raleigh, NC). Chloride was measured potentiometrically using a silver ion electrode on a salt analyzer (SAT-500, Tokyo, Japan). Lactose was determined enzymatically using a lactose assay kit (Abcam, Cambridge, MA). Protein and non protein nitrogen were analyzed by Kjeldahl (AOAC, 2000; methods 991.20;33.2.11 and 991.21;33.2.12). A conversion factor of N x 6.38 was used to determine total protein and non protein nitrogen values. Duplicate measurements were taken on each permeate for all analyses.

Organic acids

Organic acids were extracted using a modified method of Lues et al. (1998). All permeates were analyzed at 20% solids (low solid samples were measured as is and then volume was adjusted to make up for difference in solids). Sulfuric acid (20 mL, 0.013N) (Mallinckrodt Chemicals, Hazelwood, MO) was added to a 50 ml centrifuge tube (Nalgene, Rochester, NY). Sample (10 mL) was then added to this volume and vortexed and centrifuged at 7000 x G for 5 min at 4°C(Model RC5B, Thermo Scientific, Waltham, MA). The supernatant was then removed and filtered using a 0.45µm nylon syringe filter (VWR International, West Chester, PA).

Organic acids were then measured by HPLC (Waters 1525 Binary Pump,Waters, Milford, MA). Sample extract (20 µl) was injected onto the HPLC cation column (Bio- Rad Aminex HPX-87H 300 x 7.8mm, Bio-Rad, Hercules, CA). An isocratic mobile phase of 0.013N sulfuric acid was used at 0.8 ml/min. Sample was sent through a photodiode array detector

(Waters 2998). A standard curve was created for each of the organic acids**.** The maxima used for calculation was 254nm. Orotic and citric acids coeluted at this wavelength, so 285nm was used for orotic acid; at this wavelength citric acid was not detected. Organic acid standards were obtained from Thermo Scientific Inc (Fisher Scientific Inc, Pittsburg, PA).

Nucleotides

Nucleotides were extracted and measured by HPLC using modified methods described by Oliveira et al. (1999). The nucleotides measured were adenosine monophosphate (AMP), guanosine monophosphate (GMP), inosine monophosphate (IMP), cytidine monophosphate (CMP), and uridine monophosphate (UMP); these nucleotides are those that are most commonly found in dairy products (Oliveira et al., 1999; Ferreira et al., 2001). Each sample was extracted in triplicate and injected onto the HPLC in duplicate. Sample (3 mL, 10% solids, low level solids were done as is) was measured into a 50 mL centrifuge tube (Nalgene, Rochester, NY). Then to this, 2 ml of 1.0M formic acid solution (VWR International, West Chester, PA) was added into the same centrifuge tube. The sample was then vortexed. After vortexing, the samples were then centrifuged at 5000 x G for 10 min at 4°C (Model RC5B, Thermo Scientific, Waltham, MA). The top layer (3 mL) was measured into a new centrifuge tube and 200µL of 1.2M potassium carbonate (Sigma Aldrich, St Louis, MO) was added. This solution was then vortexed and centrifuged again at 5000 x G. The supernatant was then filtered through a 0.2 µm cellulose acetate membrane syringe filter (VWR International).

After filtering, the sample was injected onto the HPLC (Waters). A two solvent isocratic mobile phase was used with a gradient flow rate: solvent A consisted of 5 mM of

tetrabutylammonium hydrogensulphate (TBAHS) (Fisher Scientific Inc, Pittsburg, PA) and 20 mM potassium dihydrogensulphate (Alfa Aesar, Ward Hill, MA) and solvent B 5 mM TBAHS 100 mM di-potassium hydrogen phosphate (Alfa Aesar). The gradient flow rate was as follows: 0-20 min was 0.7 ml/min, 21-25 min flow rate was increased to 1.5ml/min, 26 min the flow was decreased to 0.7 ml/min then gradually increased to 1.5 ml/min to 37 min and maintained until 44 min. 20 μL of each sample was injected (Waters 2707 Autosampler) onto the column (Spherisorb c18- s10 ODS2: 10 μm 250x4.6mm). The injector temperature was set to 4°C. Sample was sent through a photodiode array detector (Waters 2998). A standard curve was created by diluting each nucleotide (Sigma Aldrich) in water. The maxima used for calculation was 260 nm.

Volatile Compound Analysis

Volatile compounds of permeates were determined using solid phase microextraction gas chromatography mass spectrometry (SPME GC-MS). Compounds were extracted and identified using a modified method described by Campbell et al. (2013). Five grams of permeate (10% solids w/v), 10% (w/v) of NaCl, and an internal standard(2-methyl-3-heptanone in methanol at 81 mg/kg; Sigma Aldrich) were added to 20-ml amber autosampler vials with steel screw tops with silicone septa faced in Teflon (Microliter Analytical, Suwanee, GA).

Samples were injected using a CombiPal autosampler (CTC Analytics, Zwingen, Switzerland) attached to an Agilent 7820A GC with 5975 MS(Agilent Technologies Inc., Santa Clara, CA).Before exposure, samples were maintained at 5°C. SPME fibers (1-cm

divinylbenzene/carboxen/polydimethylsiloxane fiber (DVB/CAR/PDMS, Supelco, Bellefonte, PA)) were exposed for 30 min. The initial temperature at exposure was 40°C and was ramped up to 250°C at the rate of 10°C /min. A Zb-5ms column (Zb-5ms, 30 m length × 0.25 mm i.d. × 0.25 μm film thickness; Phenomenex) was used at a flow rate of 1ml/min. The purge time was 1 min. the MS transfer line was at 250°C with the quad at 150°C and the source at 250°C. Relative abundance was determined for each compound using the calculated recovery of the internal standard. Compound identities were confirmed by comparison of retention index and mass spectra against those of authentic standards.

Descriptive Analysis

Descriptive sensory analysis was conducted to characterize both aromatics and basic tastes of each permeate and basic tastes of salts and salt blend solutions (potassium chloride (KCl, magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$) (Avantor Performance Materials, Phillipsburg, NJ), and combinations of 90:10 Na:K, 75:25 Na:K, 50:50 Na:K, 25:75 Na:K, 50:40:10 Na:K:Ca, and 50:40:10 Na:K:Mg). Permeates (10% solids,w/v or tested as is for low solids) were evaluated by a trained sensory panel (n=8, 7 females,1 male, ages 24-49 y) using an established dried dairy ingredient flavor lexicon (Drake et al., 2003; Wright et al., 2009) and a universal intensity scale consistent with the Spectrum™ method. All testing was done in accordance with the NCSU Institutional Review Board for Human Subject guidelines. Liquids (tested as is for low solids or diluted to 10% w/v solids) and rehydrated powder permeates (10% w/v) or salt solutions were dispensed into 60 ml plastic cups (Solo Cup Co., Champaign, IL) with three digit codes for tasting. Salt and salt blend solutions were prepared and evaluated at threshold, 5x threshold, and 10x threshold. Each permeate or salt

solution were evaluated in duplicate by each panelist. Compusense Five version 4.8 (Compusense, Guelph, Canada) was used for data collection.

Threshold Determination

Sensory testing was conducted in compliance with the North Carolina State University Institutional Review Board for Human Subjects approval. Best estimate taste thresholds of salts and salt blends (NaCl, KCl, $MgCl_2$, $CaCl_2$) and combinations of 90:10 NaCl:KCl, 75:25 NaCl:KCl, 50:50 NaCl:KCl, 25:75 NaCl:KCl, 50:40:10 NaCl:KCl:$CaCl_2$, and 50:40:10 NaCl:KCl:$MgCl_2$) were determined. A modified method of the ASTM procedure E679-91(ASTM, 1992), an ascending forced choice method, was used. A separate threshold test was conducted for each salt and salt blend. Solutions were prepared in distilled water and were served at 21C in 60 ml coded and lidded plastic cups. The samples were presented at 7 different ascending concentrations with a step factor of 3. At each concentration the panelist received three samples: two water blanks and one with one of the salts mentioned previously.

Subjects (n=40, ages 21-50y) were instructed prior to testing. Subjects were instructed to taste every sample in each set and select the three digit coded cup they thought was different from the other two, as well as whether or not they were sure. A 3 min enforced rest occurred between each set and subjects were instructed to rinse with spring water during this time. Noseclips (Adinstruments, Colorado Springs, Co) were worn by each panelist throughout testing to ensure that differences were due to basic tastes and not aromatics. The individual best estimate threshold (BET) was determined by taking the geometric mean of the last incorrect series and the first correct series. If the subject was unsure, a correction factor was

applied (Lawless et al., 2000; Drake et al., 2007). The group BET was calculated by taking the geometric mean of the individual thresholds.

Basic Taste Response Curves

Sodium chloride (NaCl) and lactose:NaCl intensity curves were constructed based from concentrations found in permeate. Sodium chloride at concentrations of 0.03M, 0.08M,0.13M, 0 .20M, 0.35M, and 0.50M, dissolved into deionized water, were evaluated. These solutions were also evaluated with the addition of 5 and 10% lactose (Thermo Fisher Scientific Inc, Pittsburg, PA) to mimic the composition of permeates. The samples were dispensed into 60 ml three digit coded plastic cups and lidded. Sensory analysis of basic tastes was conducted using the trained panel described previously.

Model Permeates

Permeate models were constructed with potassium chloride (KCl), lactic acid, citric acid, hippuric acid, orotic acid, and urea added to NaCl:lactose solutions described previously. These were the potential contributors of salty taste. KCl was evaluated at two concentrations: 0.5M and 0.1M, the mean of the whey and reduced lactose permeates, respectively. These were added to each concentration of NaCl and NaCl:lactose previously evaluated. The solutions were than dispensed into coded plastic 60 ml soufflé cups (Solo Cup Co., Champaign, IL) and lidded. Sensory analysis of basic tastes was conducted using the trained panel described previously. This process was repeated with lactic acid at 0.01M, 1mM citric acid, 0.69mM hippuric acid, 3.84mM orotic acid, 1.78mM uric acid and 0.52M urea

(concentration averages found in permeate). After individual models were completed, a model was generated in the same process with all the salts and acids in one model. Models were prepared and evaluated in duplicate.

Proof of Concept

Two whey permeates (1 powder and 1 liquid) from different suppliers with the lowest salty taste intensities were selected to demonstrate salty taste enhancement with KCl, lactic acid, and orotic acid. The dried whey permeate was rehydrated to 10% (w/v) solids. The liquid sample was 8% solids (w/v) and was used as-is. KCl was then added at 0.1M and lactic acid

at 0.01M, (the concentrations found in models to enhance salty taste). This was than done again with KCl (0.1M), lactic (0.01M), and orotic acid (3.84mM). Solutions were dispensed into coded plastic soufflé cups and lidded. Samples were evaluated in duplicate as previously described.

Statistical Analysis

Data was analyzed using XLSTAT (Version 2010. Addinsof, New York, NY). One way analysis of variance (ANOVA) and principal component analysis were used to evaluate differences among different permeate types. Fisher's least significant difference was used as a post- hoc test. Correlation analysis was applied to evaluate relationships between individual permeate with salty taste intensity.

Results

Proximate Analysis

Permeate composition was variable (Tables 2.1, 2.2) but consistent with reported values (US Dairy Export Council, 2011). The total solids had the widest range because both liquids and powders were provided. All permeates were less than 0.5% fat. Potassium was the most abundant mineral in permeates followed by sodium. Mineral concentrations were higher in reduced lactose permeates then in whey permeates ($p<0.05$). Reduced lactose permeate is the by-product from lactose crystallization. The first step in lactose crystallization is to concentrate permeate in an evaporator. Lactose as well as minerals and other compositionalelements are also concentrated. The pH of fresh Cheddar whey is 6.4-6.7 (Girsh, 2001). The pH of whey will decrease if whey is not heat treated to inactivate lactic acid bacteria (Girsh, 2001). According to the US Dairy Export Council (2011), the pH of permeate should be between 5.7- 6.5. The permeates in this study were within this range. Protein values in permeates would be expected to be low. Permeate is the by product of ultrafiltration of whey or milk to produce a higher protein concentration, and proteins are not be permeable to the UF membrane. Non protein nitrogen (NPN) is nitrogen that is not associated with protein. NPN values were consistent with those published for whey (Moatsou et al., 2003). The majority of the NPN found in permeate is most likely urea since it is a major part of the cow diet (Huber, 1975) and is also present in milk (Shahani and Sommer, 1951).

Organic Acids and Nucleotides

The organic acids at the highest concentration across all permeates were lactic and citric acids (Table 2.3). Whey permeates were highly variable in the concentration of these acids (180 – 2000 ppm for citric acid, 140 to 1900 ppm for lactic acid). Whey permeate composition varies widely between suppliers (Gerdes, 2011) and is due to a variety of parameters from cheese type to storage and processing of the permeate. Citric acid is naturally found in milk as an end product of bovine metabolism (Marsili et al., 1981). The reduced lactose permeates had the highest concentration of citric and lactic acids ($\bar{x}$ = 4403 ppm and 6000 ppm vs $\bar{x}$ = 1110 ppm and 897 ppm, $p<0.05$, respectively). The citric acid for reduced lactose permeates was at least two times the concentration in whey permeate (2500 to 7500 ppm). Lactic acid concentrations also varied in reduced lactose permeate (4131 ppm to 10404 ppm). Citric acid in the whey permeates was similar in concentration to that of milk and whey. Mullin and Emmons (1997) reported the concentration of citric acid in milk to be 1580 ppm and in whey products greater than 1000 ppm (1040 ppm- 1580 ppm). The lactic acid values were lower in whey and milk than in permeate. Lactic acid was not detected in fluid milks and the concentration in whey was less than 500 ppm (Mullins and Emmons, 1997). Permeate concentration of lactic acid is likely higher than that of whey products because lactic acid is a small, water soluble molecule (3 carbon chain, molecule weight of 90.08 g/mol) that is able to go through the membranes during ultrafiltration and into the permeate.

Orotic, uric, and hippuric acids were also detected in whey ($\bar{x}$ = 400 ppm, 490 ppm, and 20 ppm, respectively) and reduced lactose permeate ($\bar{x}$ = 1130 ppm, 3500 ppm, and 55 ppm, respectively). Orotic acid is a precursor of nucleic acid bases and an intermediate in pyrimidine synthesis (Stryer et al., 1989). Orotic acid is found in milk. The amount of orotic acid in milk is variable upon the cows diet but ranges from 19 and 664 ppm (Robinson, 1980; Anastasi et al., 2000). This range is similar to that found in whey permeate. Uric acid is a break down product of purine nucleotides (Giesecke et al., 1994). Giesecke et al. (1994) reported the concentration of uric acid in cow milk to be 56.2 ppm but it also varied with the cows diet. The concentration in permeates was higher than that found in milk. Uric acid concentration is variable with cows diet and also permeates themselves vary greatly which could account for different concentrations in permeate than in milk. Hippuric acid was found in similar concentrations in permeate as in milk (Marsili et al., 1981).

GMP and UMP were not detected in any of the permeates. AMP, IMP, and CMP were not detected or were below detection limits in whey permeates. Reduced lactose permeate had very low concentrations of CMP (9.3 ± 3.6 ppb). GMP, UMP, IMP, and AMP were not detected in cow's milk; CMP was found in cow's milk at 0.98 mg/100ml (Ferreira, 2003). This concentration was lower than the amount in reduced lactose permeates. The concentration in reduced lactose permeates is likely higher than that of milk because reduced lactose permeate is concentrated during manufacture.

Descriptive Analysis

Consistent with volatile compound and organic acid differences, distinct flavor differences were documented between whey permeates and reduced lactose permeates (Figure 2.1).

Whey permeates were characterized by sweet aromatics (cooked/milky, buttery,and graham cracker flavors) and sweet and salty tastes. These flavors have been previously documented in fluid whey and dried whey and milk powders (Carunchia Whetstine et al., 2003, 2005; Drake et al., 2003). Whey permeates had low salty taste ($\bar{x}$ = 2.1 salty taste intensity). The reduced lactose permeates were distinctly salty ($\bar{x}$ = 9.9 for salty taste intensity) with savory aromatics: potato, beefy, and vitamin flavors, and sour and umami tastes. Salty taste was higher in whey permeates than salty taste intensities reported in fluid whey or milk (permeate:1.0-3.0 vs. fluid milk or fluid whey: 0.5-1.0) (Carunchia Whetstine et al., 2003). Potato/brothy flavor has been previously reported in fluid whey and other dried whey ingredients and has been attributed to methional (Karagul-Yuceer et al., 2002; Mahajan et al., 2004; Carunchia Whetstine et al., 2005). Beefy/brothy flavor has been documented in Cheddar cheese as well as sour cream and Greek yogurt and has been sourced to 2-methyl-3-furanthiol (Cadwallader et al., 2006; Shepherd et al., 2013; Desai et al., 2013).

Flavor differences were also documented between liquid and spray dried permeates. Liquid permeates had higher intensities of cooked/milk and buttery flavors, and sweet taste; whereas spray dried powders had higher intensities of graham cracker flavor. This suggests that flavor profiles are altered during the spray drying process. Whitson et al. (2011) reported that spray dried whey concentrate and isolate was more intense in flavor than liquid whey protein retentate and suggested that this difference was due to spray drying. Cardboard flavor was increased in spray dried product compared to liquid retentate. Maillard browning reactions also occur during spray draying and may contribute to flavors (Ferretti and Flanagan, 1971;1972; Labuza and Saltmarch,1981; O'Brien and Morrissey, 1989).

Volatile Compounds

Similar volatiles were found in permeates that have been reported in fluid whey and other dairy dried whey products such as whey protein concentrate and sweet whey powders (Carunchia Whetstine et al., 2005; Mahajan et al., 2004; Karagul- Yuceer et al., 2002; Carunchia- Whetstine et al., 2003)(Tables 2.5-2.6). The concentration of volatiles in permeates was lower than values reported in fluid and dried whey ingredients. These volatiles included aldehydes, sulfur containing compounds, and diacteyl (Mahajan et al., 2004; Carunchia Whetstine et al., 2003). Sulfur containing compounds such as DMDS and methional are Strecker degradation products of methionine (Tressl et al., 1989; Ballance, 1961). DMDS has a cabbage aroma and methional has a potato aroma (Carunchia Whetstine et al., 2005). Aldehydes were found in all permeate types and included pentanal, hexanal, heptenal, and nonanal. Aldehydes are the result of lipid oxidation (Frankel, 1982). While present in all samples, the concentration of aldehydes was highest in the reduced lactose samples. Aldehydes in many dairy products increase with heat and storage (Shiratsuchi et al., 1994; Contarini et al., 1997; Vazquez- Landaverde et al., 2005; Whitson et al., 2011). Ultra pasteurized milk had a higher concentration of aldehydes present then either raw milk and pasteurized milk (Contarini and Povolo, 2002; Vazquez- Landaverde et al., 2005). Liquid ultra pasteurized milk had a higher concentration of aldehydes present then either raw milk and pasteurized milk (Contarini and Povolo, 2002; Vazquez- Landaverde et al., 2005). Liquid WPC and WPI retentates increased in hexanal and heptanal with increased storage (Whitson et al., 2011). Reduced lactose permeate are concentrated through evaporation and are

exposed to a high heat load and additional storage and process time, which likely increases the aldehydes that are present.

Pentanal, heptanal, nonanal, and sulfur compounds are sources of cardboard flavor in whey ingredients (Whitson et al., 2010). Two of the reduced lactose permeates had cardboard flavor. These two permeates also had higher amounts of these aldehydes. Reduced lactose permeates had higher concentration of volatiles present and also had different volatiles than milk or whey permeates (Figure 2.2). Reduced lactose permeates were characterized by 2-methyl-3-furanthiol, heptanal, nonanal, hexanal, and p-cresol and these are indicative of longer storage/ process time and heat treatment. 2-methyl- 3- furanthiol has a meaty flavor (Carunchia Whetstine et al., 2005) and p-cresol is commonly thought to be urine like or barny (Bendall, 2001; Kim et al., 2003). These results coincide with the sensory profiles of reduced lactose permeates which were more savory and less sweet than whey or milk permeates.

Diacetyl was present in all permeates (Tables 2.5-2.6). Whey permeate had the highest concentration of diacetyl. Diacetyl is produced by bacteria during cheese manufacture and is known for its buttery/ sweet flavor (Mahajan et al., 2003; Bendall, 2001; Kieronczyk et al., 2003). Because diacetyl is a byproduct of fermentation it was expected that whey permeates would be higher in this volatile than milk; although, diacetyl is also found in raw milk at low concentrations (Karagul-Yuceer et al., 2002; Carunchia Whetstine et al., 2003). Whey permeates were characterized more by diacetyl than reduced lactose permeates. Reduced lactose permeate goes through a period of heat treatment for evaporation and crystallization.

Islam and Langish (2010) found that higher spray drying and crystallization temperatures increased lactose yields. Diacetyl is lowered by heat; the boiling point of diacetyl is about 88°C (Burdock, 2010). pH is also a factor in reduced lactose permeates because it affects the crystallization of lactose (Guu and Zall, 1991). Diacetyl is produced at maximum at a pH of 5.5 (Cachon and Divies, 1993; Cachon and Divies, 1994). The pH range for reduced lactose permeates was below this ideal pH and combined with the heat treatment effects could explain the lowered concentration of diacetyl in reduced lactose permeate. Liquid permeates had a higher concentration of diacetyl than dried permeates and may be due to volatilization of diacetyl during the spray drying process.

Sensory thresholds and basic tastes of salts and blends

Threshold values in this study were KCl>$CaCl_2$>$MgCl_2$ >NaCl (Table 2.7). These thresholds were consistent with values reported in other studies (van Gemert, 2003). Thresholds of NaCl: KCl blends and other salt combinations have not been previously published. Blends had threshold values between the two compounds alone. NaCl: KCl (90:10) was most similar in threshold value to pure NaCl (115 ppm for blend vs. 127 ppm for pure salt). Other NaCl:KCl blends had similar threshold concentration (221 ppm-247 ppm).

NaCl, KCl, $MgCl_2$, and $CaCl_2$ all displayed salty taste at threshold levels (Table 2.8). KCl was the only salt solution that exhibited bitter taste at threshold concentration. KCl has been studied extensively and bitter taste is commonly reported (McCaughey, 2007; Beauchampand Stein, 2008; Sinopoli and Lawless, 2012). NaCl and mixtures of NaCl and KCl had the highest salty taste intensities. As the concentration was increased to 5x

threshold,the salt solution with the highest salty taste was the blend of NaCl:KCl 75:25. This was followed in salty taste intensity by NaCl, KCl, and a 90:10 blend of NaCl:KCl. At 5x threshold, $MgCl_2$ and $CaCl_2$, also had a bitter taste (Table 2.8). The salt with the highest intensity for salty taste at 10x concentration was the 75:25 blend of NaCl:KCl. NaCl:KCl 75:25 did not have any bitter taste associated with it. Other studies reported that NaCl will decrease the bitter taste detected in KCl when blended (Keast and Hayes, 2011).

Sources of salty taste

The compositional elements with the signifcant correlation values to salty taste intensity were KCl, lactic acid, citric acid, orotic, and non protein nitrogen (Table 2.9.) Elements with significant correlation values ($p<0.05$) were evaluated in permeate models to determine their effect on salty taste.

Basic Taste Response Curves/ Model Permeates

NaCl, both without added lactose and with lactose, had lower salty taste intensity scores than whey permeate and reduced lactose permeate ($\bar{x}$ =2.1 and 10.5, respectively) at similar concentrations of NaCl (Figure 2.3). The addition of lactose decreased salty taste intensity of NaCl solutions. Sweet taste suppresses salty taste (Pangborn, 1959). Permeates elicited a higher salty taste response than the same concentration of NaCl alone suggesting other components of permeate contribute to salty taste. The reduced lactose permeates had the highest salty taste intensity which would be expected since minerals and organic acids were concentrated. Potassium chloride, lactic acid, citric acid, orotic acid, hippuric acid, uric acid, and urea were selected for creation of permeate models. These compounds were selected

because they had a significant correlation with salty taste (Table 2.9). When potassium (KCl) was added to NaCl at 0.05M, the concentration found within the permeates, the perceived salty taste intensity increased in the presence or absence of lactose (Figure 2.4). Other research has also reported that the partial substitution of KCl with NaCl enhanced salty taste and also reduced the bitterness of KCl alone (Keast and Hayes, 2011). Whey permeates fall above the salty taste intensity for NaCl alone but when KCl was added to permeate models, the salty taste intensity of the models was the same intensity as whey permeates ($\bar{x}$ =2.1 salty taste intensity) (Figure 2.4). The second KCl curve included the highest concentration of KCl that was found in the reduced lactose permeates. The increased concentration of KCl yielded a greater salty taste intensity than just that of NaCl alone or that of the lower concentration of KCl (Figure 2.5).

The addition of 0.01M lactic acid to NaCl also enhanced the perceived salty taste (Figure 2.6). Addition of lactic acid to NaCl increased salty taste intensities ($p<0.05$). Lactic acid and NaCl with and without lactose raised salty taste intensities to a similar intensity of salty taste that was found in whey permeates ($\bar{x}$ =2.1). Citric acid and NaCl was higher in salty taste intensity than NaCl alone but with the addition of lactose, the salty taste intensity was similar to those of NaCl. All permeate types had a higher salty taste intensity than citric acid and NaCl or NaCl alone (Figure 2.7). Orotic acid increased salty taste intensities (Figure 2.8). Uric acid, hippuric acid, or urea did not increase the salty taste of NaCl solutions nor were these compounds salty tasting ($p>0.05$) (data not shown). Uric acid was sour and urea was bitter at the mean concentration detected in permeates. Hippuric acid concentrations in permeates were below sensory threshold.

NaCl was combined with KCl and lactose and each acid to determine if the addition of acid increased salty taste more than KCl alone (Figure 2.9). Citric and hippuric acids did not increase salty intensity more than KCl and NaCl ($p>0.05$). Lactic and orotic acids increased salty taste intensity of KCl and NaCl ($p<0.05$). With the addition of lactic or orotic acids, the salty taste intensity was more than the individual elements alone (Figure 2.9). This result suggests that KCl is not the only factor increasing the salty taste in permeates. The addition of lactic acid and orotic acid to KCl and NaCl increased the salty taste intensity to the intensity of whey permeates. Because reduced lactose permeate is less than 5% lactose in solution, it was still higher in salty taste intensity than the salty taste intensity of the model permeate. The salty taste intensity of permeate models with KCl, lactic acid, orotic added to NaCl suggested that these compounds were also sources of salty taste in permeate.

To determine if salty taste enhancement was from KCl and lactic acid or if other compounds were also enhancing salty taste, three models were constructed (Figure 2.10). The first (model 1) was KCl, lactic acid, lactose, and NaCl. The second (model 2) included elements from this study that increased the salty taste intensity of NaCl: this model included KCl, lactic acid, lactose, and orotic acid. The third (model 3) included all elements that were correlated with salty taste and lactose: KCl, lactic acid, citric acid, hippuric acid, orotic acid, hippuric acid, uric acid, and urea. All of the models increased salty taste intensity from NaCl alone (Figure 2.10). Model 2 had higher salty taste intensity than model one, suggesting that other elements beside KCL and lactic acid increased salty taste. Model 2 was not different in salty taste from model 3. This suggests that orotic acid also increased salty taste intensity

since it was absent in model one but that the addition of other acids and urea did not further increase salty taste. Finally, KCl and lactic acid and KCl, lactic acid and orotic acid at

concentrations that were evaluated in the model systems were added to two commercial whey permeates with low salty taste intensities ($\bar{x}$ =1.8 and 0.5). With the addition of 0.1M KCL and 0.01M lactic acid, salty taste intensities of the low salty taste whey permeates increased ($p<0.05$) (0.5 increased to 7 and 1.6 increased to 7.5). With the addition of 0.1M KCl and 0.01M lactic acid and 3.84mM orotic acid, salty taste intensities of the low salty taste whey permeates increased ($p<0.05$) (0.5 increased to 7.7 and 1.6 increased to 8.6) and these values were higher ($p<0.05$) than 0.1M KCl and 0.01M lactic acid alone.

Conclusions

Elements in permeate interact to provide an enhancement of salty taste. Lactic acid and KCl have the greatest enhancement effect in permeate, but orotic acid can also provide salty taste enhancement. Less sodium could be added using permeate with the same salty taste intensity as using a higher concentration of sodium. Reduced lactose permeate has the highest concentration of these compounds and would best be suited as a replacement of sodium in food products. Reduced lactose permeate also had the highest perceived salty taste intensity. In order to maximize whey or milk permeates potential as a salt replacer, the product should have higher concentrations of both lactic acid and KCl. One potential way to increase lactic acid would be to start with acidified whey. If lactic acid is at a higher concentration initially in fluid whey, more will be present in the permeate following membrane fractionation. Also, nanofilteration could be used to separate the minerals from the lactose. Lactose has a

suppressive effect on salty taste, so if minerals were removed from fluid whey directly with lactose they could enhance salty taste to a greater degree.

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TABLES

Table 2.1 Proximate analysis averages of all permeate types.

Sample Type		Total solids (%)	pH	Lactose (% dry weight)	True Protein (% dry weight)	NPN (%) (% dry weight)
Whey permeate (n=14)	Average	54.1	6.15	85	0.03	3.31
	Range	(2.7-98.8)	(5.9-6.8)	(84-86)	(ND-0.09)	(2.61-4.08)
Reduced lactose permeate (n=4)	Average	66.4	5.8	46.5	0.19	7.69
	Range	(32.5-98.5)	(5.0-6.6)	(45-50)	(ND-0.88)	(6.13-9.55)

Ranges of all sampled permeates are provided in parenthesis. Some whey permeates were in powder form (n=6) and others were liquids (n=8). True protein is the actual amount of protein in sample (total protein-npn).ND-Not detected.

Table 2.2 Average mineral concentration in permeates.

Sample Type		%K	%Ca	%Mg	%Na	%Cl	Ash(%)
Whey permeate	Average	2.13	0.54	0.12	0.98	0.21	8.26
	Range	(1.54-3.48)	(0.36-0.86)	(0.11-0.14)	(0.70-1.33)	(0.07-0.67)	(5.28-11.8)
Reduced lactose permeate	Average	6.04	1.51	0.25	2.39	1.03	21.8
	Range	(4.24-7.38)	(0.43-3.55)	(0.21-0.27)	(1.68-3.50)	(0.79-1.79)	(20.7-22.9)

Ranges of all sampled permeates are provided in parenthesis. % dry weight

Table 2.3 Organic acid concentrations in permeates

Sample	Citric	Orotic	Lactic	Uric	Hippuric
Whey permeates					
S1	1770d	543ef	1896de	227e	12efg
S2	1943d	584de	1096ef	321e	12efg
S3	1837d	781cd	1422ef	644de	124a
S4	1795d	685de	1517ef	377de	14defg
S5a	1939d	643de	966ef	267e	14def
S5b	2049d	639de	1614ef	299e	14def
S6	2203cd	755cde	118f	1960c	29c
S7a	1176de	283fg	1149ef	992d	17cdef
S7b	1651de	247g	902ef	1043d	27c
S8	152e	69g	117f	178e	ND
S9	42e	58g	203ef	27e	ND
S10	123e	63g	140f	28e	ND
S11	671de	142g	522ef	331de	ND
S12	180e	63g	906ef	200	ND
Reduced lactose permeate					
S1	2528bcd	1292b	6586b	1034d	29c
S2	3434bc	622de	2918cd	492de	21cd
S3	7478a	1000bc	4131cd	4858d	46b
S4	4173b	1618a	10404a	7762a	124a

S-supplier, a and b denote different products by same supplier, in ppm, different lettering within a column for each permeate type is significantly different ($p < 0.05$).

Table 2.4 Trained panel sensory profiles of permeates

Sample	Aroma	buttery	Cooked/milky	Cardboard	Potato	Beefy	Graham Cracker	vitamin	Sweet	Sour	Salty	Bitter	umami	astringent
							Whey permeate							
S1	1.5def	ND	1.3de	1.3abc	0.8cd	0.6b	1.0ab	ND	2.3abc	1.0a	3.3abc	ND	2.5ab	ND
S2	1.5def	ND	1.5ef	1.0c	ND	0.8ab	1.0ab	ND	2.6ab	1.2a	2.2cd	ND	2.4ab	ND
S3	2.0cd	ND	1.0c	1.4bc	0.5d	1.1a	1.1ab	ND	2.5ab	1.1a	2.4de	ND	2.3ab	ND
S4	1.4ef	ND	1.1d	0.6c	0.5d	1.0ab	1.3ab	ND	2.2b	2.0a	3.0b	ND	3.0a	ND
S5a	2.2de	ND	0.5f	1.6a	1.6ab	1.0ab	0.8b	ND	2.2b	1.6a	1.8fg	ND	2.3ab	ND
S5b	2.3ab	ND	0.8f	1.5ab	1.3b	1.1a	1.8a	ND	2.4ab	1.5	2.0fg	0.5b	2.1bc	ND
S6	3.1a	3.1a	2.3bc	ND	2.5a	ND	ND	ND	1.5fg	ND	1.2ghi	0.8ab	1.0de	ND
S7a	1.0f	1.0	1.9c	0.8bc	0.8cd	ND	1.1ab	ND	2.8a	ND	0.9hi	ND	1.3cd	ND
S7b	3.0ab	3.0a	2.5bc	0.8bc	1.3b	ND	ND	ND	1.6efg	ND	1.4gh	ND	1de	ND
S8	2.5abc	2.5ab	2.8ab	1.2abc	ND	ND	ND	0.5a	1.6efg	1.0a	1.2ghi	1.0a	1.4cd	1.5a
S9	2.3bcd	2.3ab	1.3de	1.3abc	ND	ND	1.9a	ND	2.2bcde	ND	1.2ghi	ND	1.3cd	ND
S10	1.5def	1.5c	2.3bc	0.5c	0.5d	ND	1.0ab	ND	2.2bcde	ND	1.3gh	ND	1.0de	ND
S11	2.0cde	2.0bc	2.3bc	1.0abc	0.5d	ND	ND	ND	1.8defg	1.0a	4.3a	ND	2.1bc	ND
S12	2.8abc	2.8ab	3.3a	ND	ND	ND	ND	ND	1.2g	ND	0.5i	ND	0.5e	1.3a
							Reduced lactose permeate							
S1	3.3b	ND	ND	ND	2.5a	2.0a	ND	ND	1.3bc	3.7a	10.8b	1.4a	4.8a	ND
S2	1.6c	ND	2.0a	1.5b	ND	ND	0.9a	ND	2.5b	0.4c	8.0c	0.5b	3.3b	ND
S3	3.5a	3.5a	ND	2.2a	1.3b	ND	ND	3.2a	3.2a	1.6b	12.8a	ND	2.8c	ND
S4	3.6a	3.6a	ND	2.1ab	1.8ab	ND	ND	2.3b	0.7c	2.8ab	8.1c	ND	3.3b	ND

S- supplier, a and b denote different products by the same supplier. A 0 to 15 point universal scale was used for sensory intensities. Different lettering within each column within each section indicates significant differences ($p < 0.05$).

Table 2.5 Volatile compounds of whey permeates

	S1	S2	S3	S4	S5a	S5b	S6	S7a	S7b	S8	S9	S10	S11	S12
Diacetyl	12.0cd	5.50def	0.87f	1.35f	0.93f	8.50de	20.0bc	17.0bc	33.0a	17.0bc	5.10ef	4.80ef	19.0b	5.85def
1-hexen-3-one	5.48c	5.06c	0.77gh	1.99efg	4.13cd	5.04c	4.00cd	4.28cd	8.20b	5.34c	2.92ef	3.00ef	4.83c	0.13h
1-pentanol	5.47d	2.40def	1.83ef	1.74ef	3.13def	2.97def	2.90ef	13.2c	3.58de	5.34d	2.29def	2.20def	1.11ef	0.12f
DMDS	0.08d	0.14c	0.06de	0.05ef	0.04efg	0.16bc	0.02fghi	0.03fgh	0.18b	0.03fgh	0.800a	0.80a	0.04efgh	0.02ghi
2-methyl-3-furanthiol	0.06c	0.03c	0.01c	0.02c	0.01c	0.05c	0.03fghi	0.03fgh	0.07c	0.10c	0.03c	0.02c	0.05c	0.01c
benzaldehyde	7.10ab	1.71def	3.51cde	4.73bc	1.59def	2.64cdef	1.10def	1.12def	0.57f	4.92bc	3.00cdef	3.00cdef	0.85ef	0.66f
4-octanone	6.91ef	4.25ghi	4.40ghi	3.82hi	4.23ghi	5.78fg	7.00ef	14.1d	8.54e	7.15ef	8.72e	8.50e	4.74ghi	21.6c
p-cresol	0.03g	0.06f	0.02g	0.03g	0.10de	0.50f	0.09e	0.12d	0.09e	0.13c	0.02g	0.02g	0.09e	0.01h
2,6-nonadienal	0.29b	0.21b	0.29b	0.18b	0.16b	0.25b	0.02b	0.23b	0.25b	0.61b	0.43b	0.43b	0.31b	0.20b
DMS	4.55ab	4.26ab	0.96c	1.79abc	3.21abc	2.61abc	3.50abc	3.58bc	3.68abc	0.71c	5.10ef	1.5bc	19.0b	5.85def
Pentanal	3.59cde	1.38gh	1.97efgh	1.99efg	3.27cdef	3.36cdef	3.00defg	2.32b	3.83cd	5.82ab	2.65defgh	2.40defg	1.73fgh	1.10h
Hexanal	3.02fgh	1.84gh	3.92efg	1.94gh	0.89h	7.86cd	5.57bc	5.79bc	10.28b	2.35fgh	2.45fgh	2.35fgh	4.34ef	1.27h
Methional	0.02bc	0.02cde	ND	0.01defgh	0.01efgh	0.02cd	0.01ijk	0.01cdefg	0.04a	0.01b	ND	ND	0.01cdefg	ND
Heptanal	4.29hijk	18.3efg	13.2fghi	12.2fghji	26.3e	19.4efg	3.00defg	125a	37d	2.22jk	2.70ijk	2.60ijk	14.4fgh	0.30k
Nonanal	0.52g	0.94fg	1.13efg	0.92fg	1.13efg	1.97de	3.00defg	4.74c	2.59d	4.87c	1.02efg	1.00efg	1.00fg	1.50ef

S- Supplier, a and b denote different products by the same supplier, different lettering within a row indicates significant differences ($p < 0.05$), values in p

Table 2.6 Volatile compounds of reduced lactose permeates

	S1	S2	S3	S4
Diacetyl	1.50c	16.0a	4.15bc	8.05b
1-hexen-3-one	2.16b	10.9a	1.78b	11.03a
1-pentanol	0.89c	100a	1.95c	35.01b
DMDS	0.04b	0.18a	0.02bc	0.01c
2-methyl-3-furanthiol	0.02c	3.11a	0.01c	2.28b
benzaldehyde	8.49a	1.28c	1.29c	7.04b
4-octanone	3.28c	44.7a	5.62c	32.6b
p-cresol	0.02d	0.16b	0.06c	0.27a
2,6-nonadienal	0.46b	11.3a	0.18b	14.1a
DMS	2.50a	2.99a	2.12a	4.895a
Pentanal	7.33a	3.14c	5.80ab	4.83bc
Hexanal	2.16b	281a	5.79b	5.40b
Methional	0.02cde	0.03b	ND	0.01cdefgh
Heptanal	7.12c	580a	21.6c	293b
Nonanal	0.84c	13.0a	1.45c	11.5b

S- supplier, different lettering within column is significantly different ($p<0.05$), values in ppb

Table 2.7 Best estimate thresholds of different ratios of potassium chloride (K), magnesium chloride (Mg), calcium chloride (Ca), and sodium chloride (Na) salts in water.

Salt(s)	Ratio (%)	Threshold Value (ppm[1])	Threshold Value (mM)	Standard Deviation (ppm)
K	100	390	5.23	28
Mg	100	137	1.44	34
Ca	100	237	2.15	42
Na	100	127	2.17	20
Na:K	90:10	115	1.91	22
Na:K	75:25	244	3.91	34
Na:K	50:50	247	3.39	21
Na:K	25:75	221	3.13	34
Na:K:Ca	50:40:10	187	2.67	19
Na:K:Mg	50:40:10	225	3.29	27

[1]*ppm=parts per million, Thresholds represent the geometric means from 40 consumers*

Table 2.8 Salty and bitter taste intensities of salts and salt blends.

		1x		5x		10x	
Salt	**ratio**	**salty**	**bitter**	**salty**	**bitter**	**salty**	**bitter**
KCl	100	0.6a	1.1a	0.9acd	1.3a	1.5abc	1.6b
MgCl	100	0.5a	ND	0.6e	0.7c	0.7f	0.8c
CaCl	100	0.5a	ND	0.7d	1.0ab	0.80ef	2.1a
NaCl	100	0.5a	ND	1.0bc	ND	1.3bcd	ND
Na:KCl	90:10	0.5a	ND	1.0b	ND	1.8a	ND
Na:KCl	75:25	ND	ND	1.5a	ND	2.0ab	ND
NA:KCl	50:50	ND	ND	1.0cd	ND	1.5ab	ND
Na:KCl	25:75	ND	ND	1.7cd	ND	2.0def	0.9c
Na:K:Mg	50:40:10	ND	ND	1.0cd	ND	1.2cde	ND
NA:K:Ca	50:40:10	ND	ND	0.8cd	ND	1.3bcd	ND

Based on mean best estimate threshold in this study, 1x- threshold, 5x- 5 times threshold, and 10x- ten times threshold respectively. ND- not detected. Metallic taste was detected in all salt blends at intensities of 0.5 for 5x and 1.0 for 10x. Intensities are based on a 0 to 15 point universal scale.

Table 2.9 Correlation between permeate compositional elements and salty taste of permeate

	%K	**%Ca**	**%Mg**	**%NPN**	**Citric**	**Orotic**	**lactic**	**Uric**	**hippuric**
r^2 value	0.87	0.02	0.09	0.62	0.61	0.55	0.77	0.55	0.54
p value	<0.0001	>0.05	>0.05	<0.0001	<0.0001	<0.0001	<0.0001	>0.05	>0.05

FIGURES

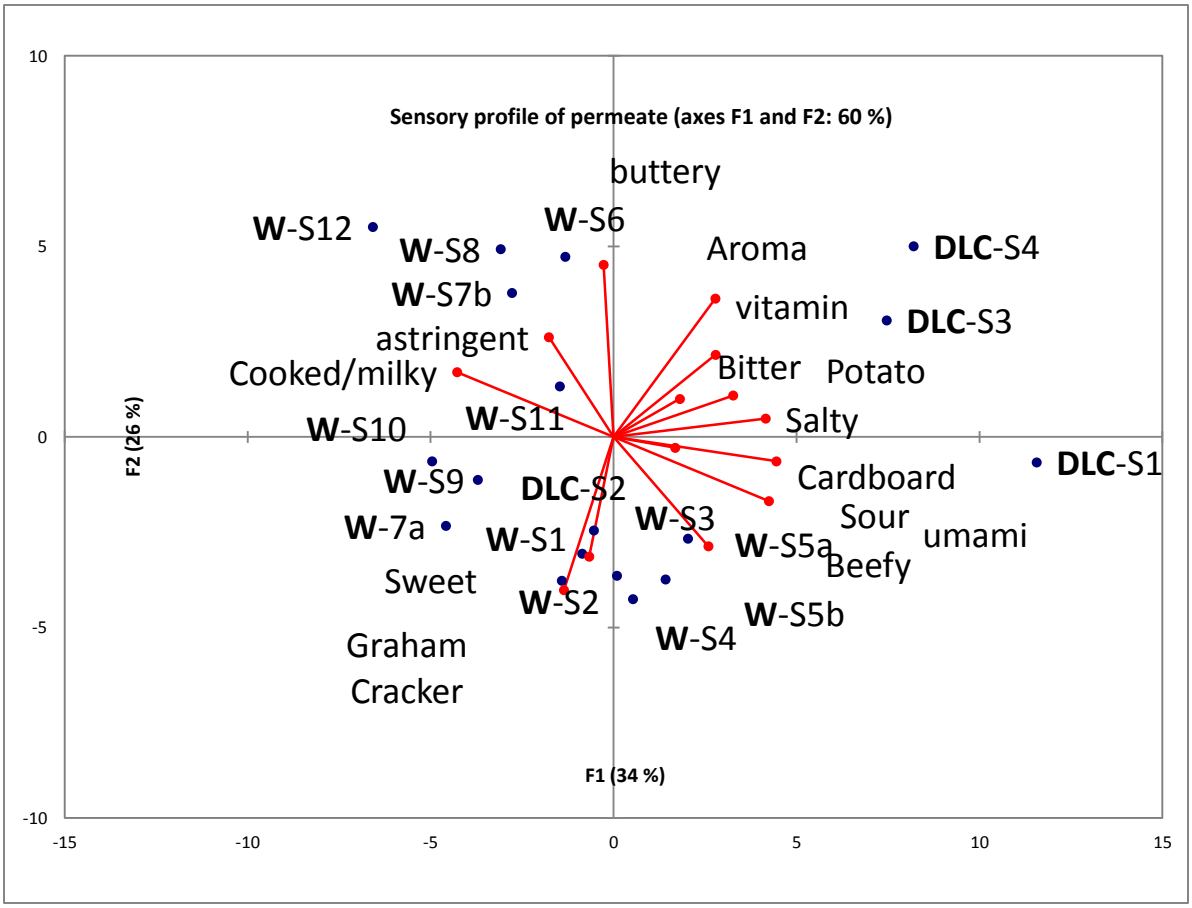

Figure 2.1 Principal component biplot of sensory profiles of permeates ***W-*** *whey permeates,* ***DLC*** *– reduced lactose permeate, S- supplier, a and b represent same supplier but different product*

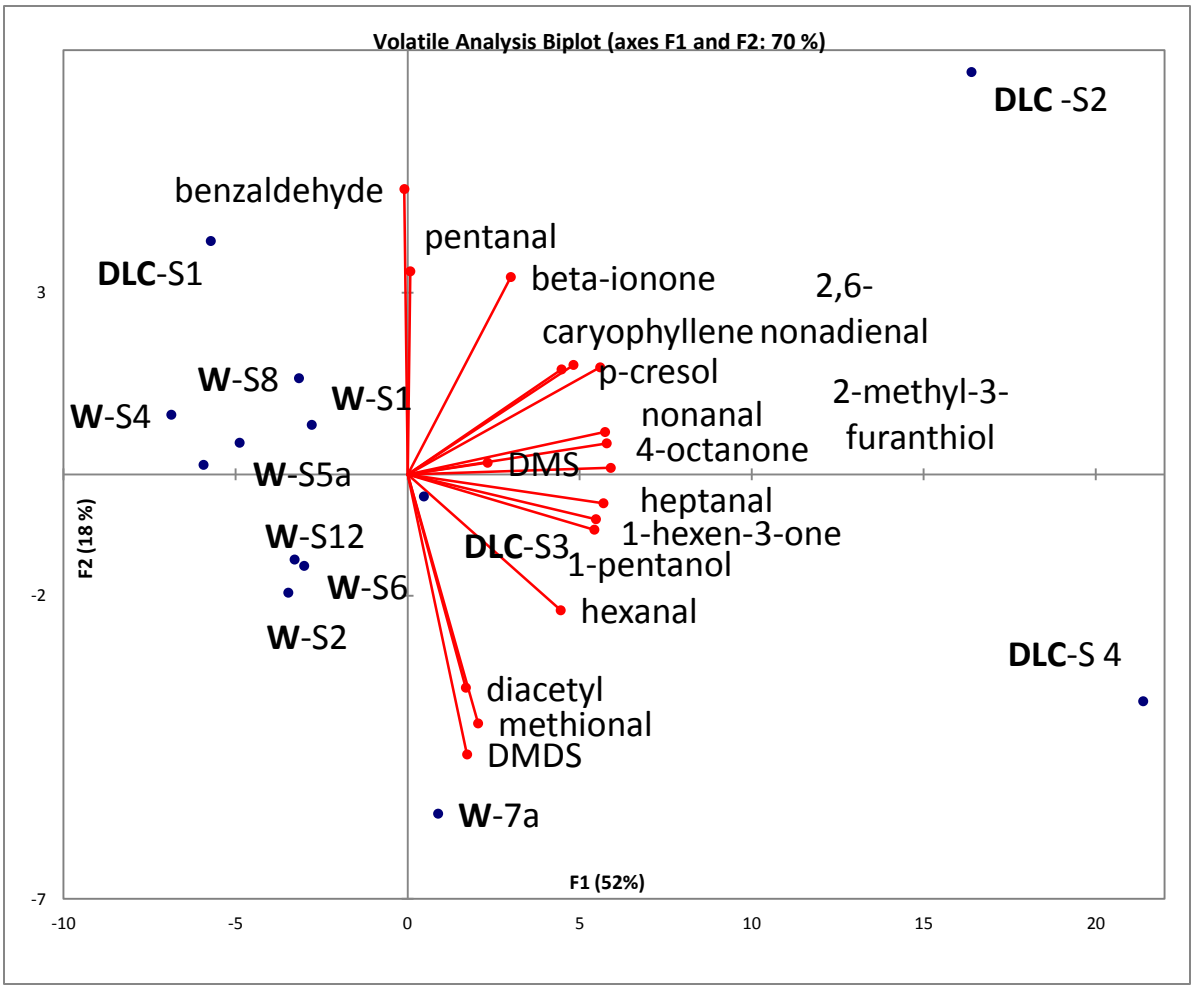

Figure 2.2 Principal component biplot of volatile compounds in permeate and reduced lactose permeates (ppb)*W- whey permeates, DLC – reduced lactose permeate, S- supplier, a and b represent same supplier but different products*

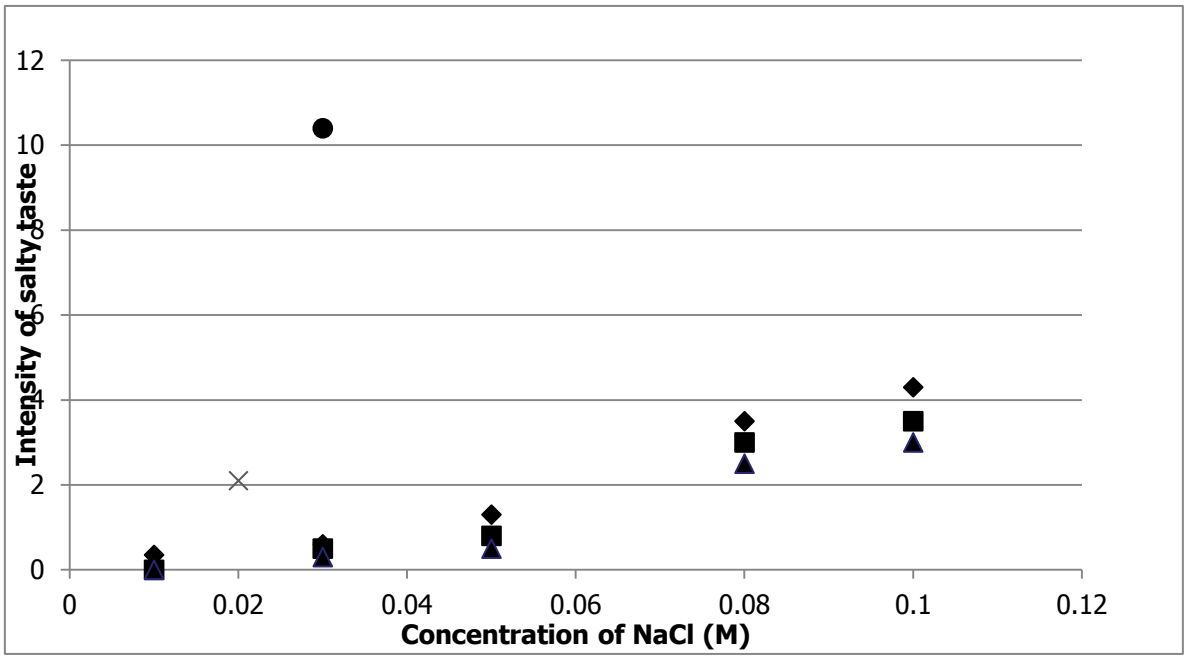

Figure 2.3 Salty taste response curves of permeate and NaCl:lactose solutions *(♦)- NaCl and no lactose, (■)- NaCl and 5% lactose, (▲)- NaCl and 10% lactose, (×)- Mean of whey permeate,(●)- mean of reduced lactose permeate, 0 to 15 point universal scale used for intensities*

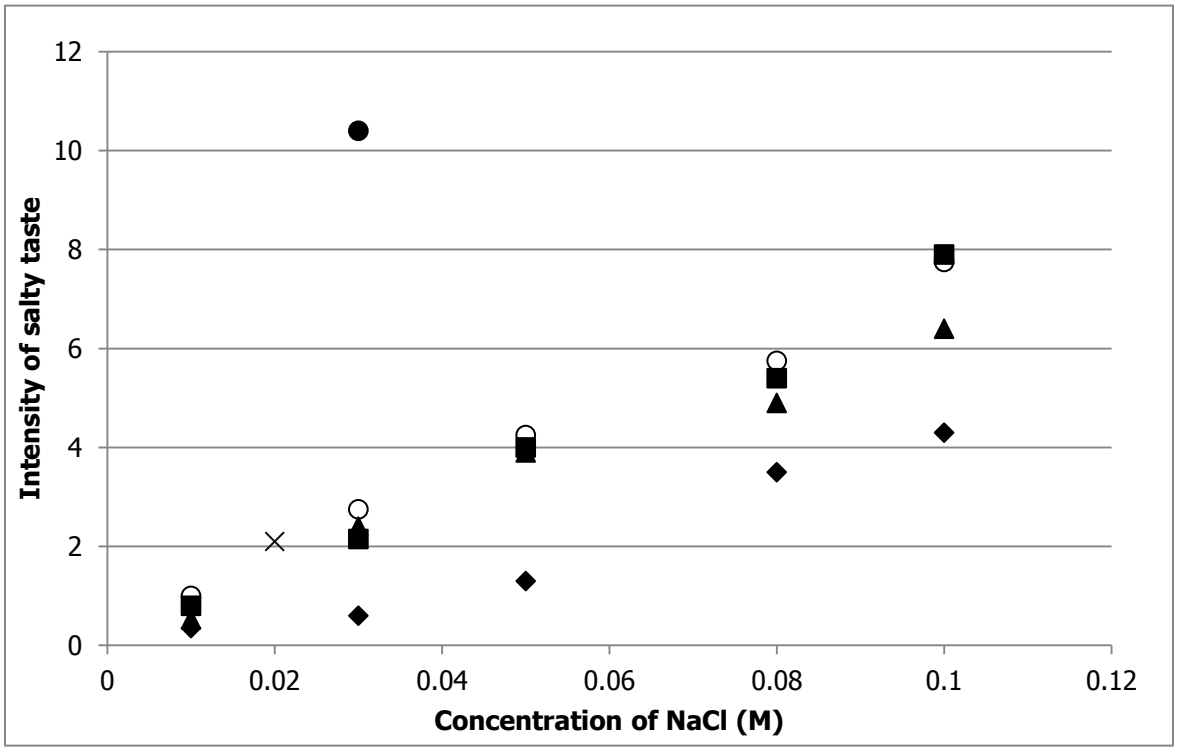

Figure 2.4 Salty taste response curves of NaCl and 0.05M KCl with and without lactose*(♦)-NaCl and no lactose,(○)- NaCl+KCl, no lactose (■)- NaCl,KCl and 5% lactose, (▲)- NaCl, KCl and 10% lactose, (×)- Mean of whey permeate, (●)-Mean of reduced lactose permeate. 0 to 15 point universal scale used for intensities*

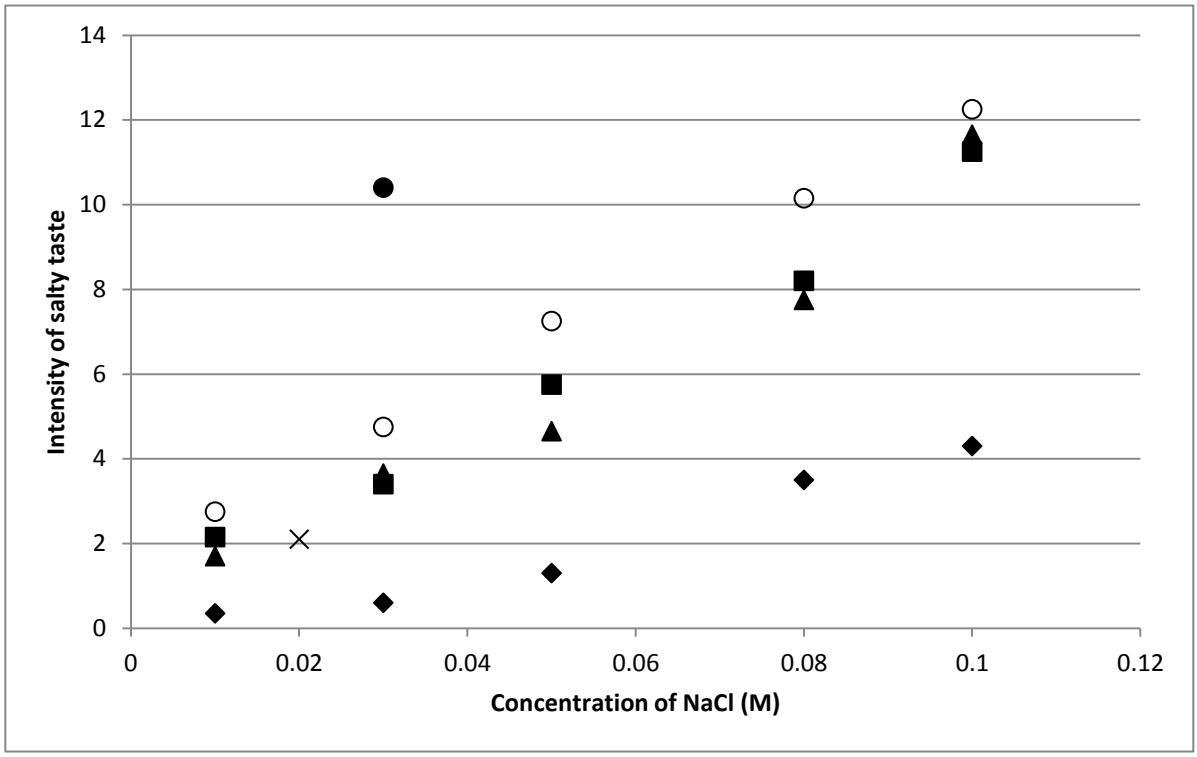

Figure 2.5 Salty taste response curve of NaCl and 0.10M KCl with and without lactose*(♦)- NaCl and no lactose,(○)- NaCl+KCl, and no lactose (■)- NaCl, KCl and 5% lactose, (▲)- NaCl, KCl , and 10% lactose, (×)-Mean of whey permeate, (●)-Mean of reduced lactose permeate. 0 to 15 point universal scale used for intensities*

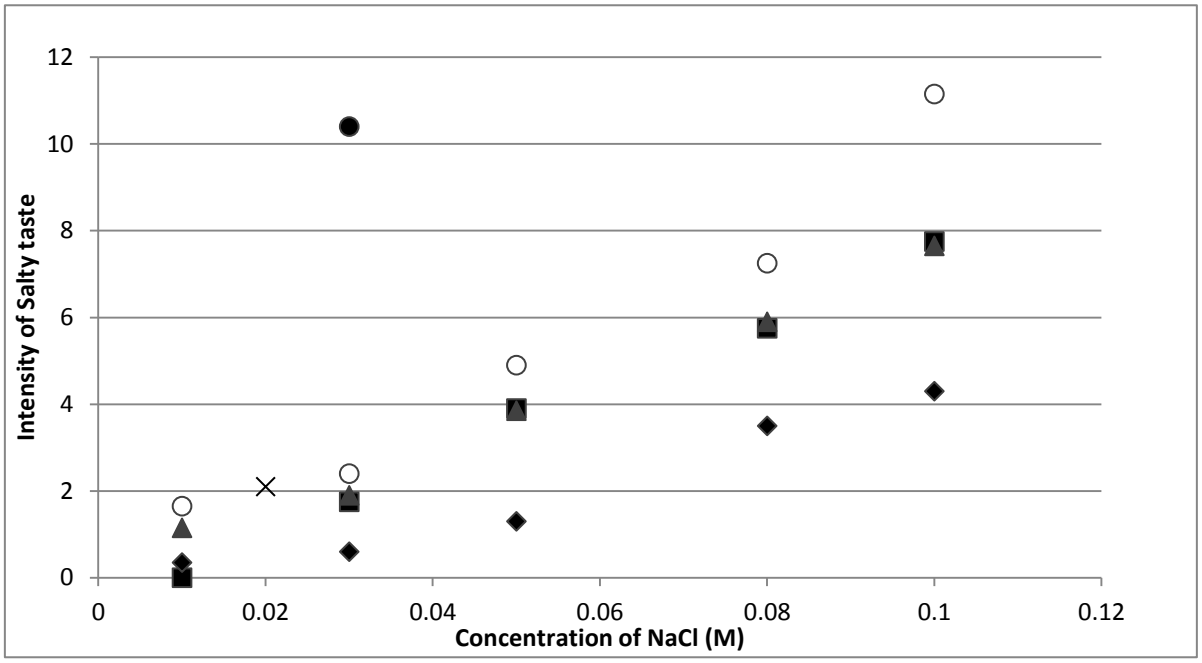

Figure 2.6 Salty taste response curves of NaCl and0.01M lactic acid with and without lactose(♦)- NaCl,(○)- NaCl+lactic acid, no lactose (■)- NaCl, lactic acid, and 5% lactose, (▲)- NaCl, lactic acid, and 10% lactose, (×)- Mean whey permeate,(●)-Mean of reduced lactose permeate. 0 to 15 point universal scale used for intensities

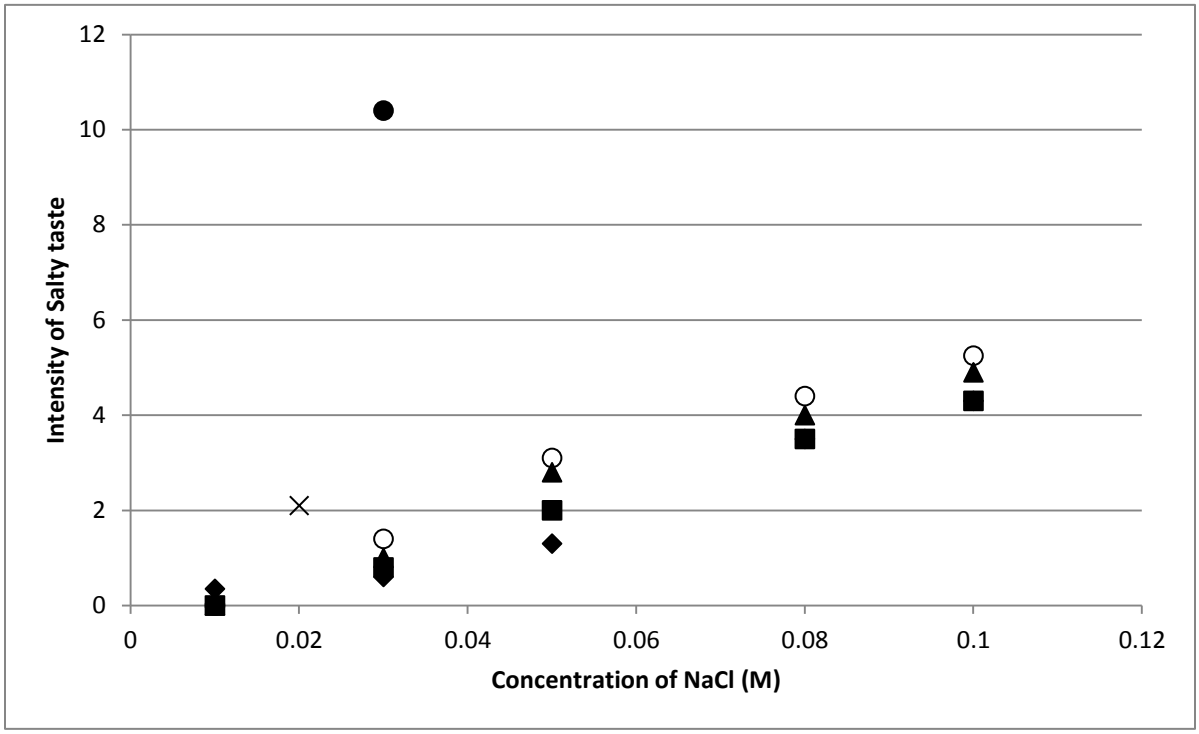

Figure 2.7 Salty taste response curves of NaCl and 1mM citric acid with and without lactose(♦)- *NaCl and no lactose,(○)- NaCl,Citric acid, no lactose,(■)-Nacl, Citric acid, and 5% lactose,(▲)- NaCl, citric acid, and 10% lactose, (×)- Mean of whey permeate,(●)- Mean of reduced lactose permeate. 0 to 15 point universal scale used for intensities*

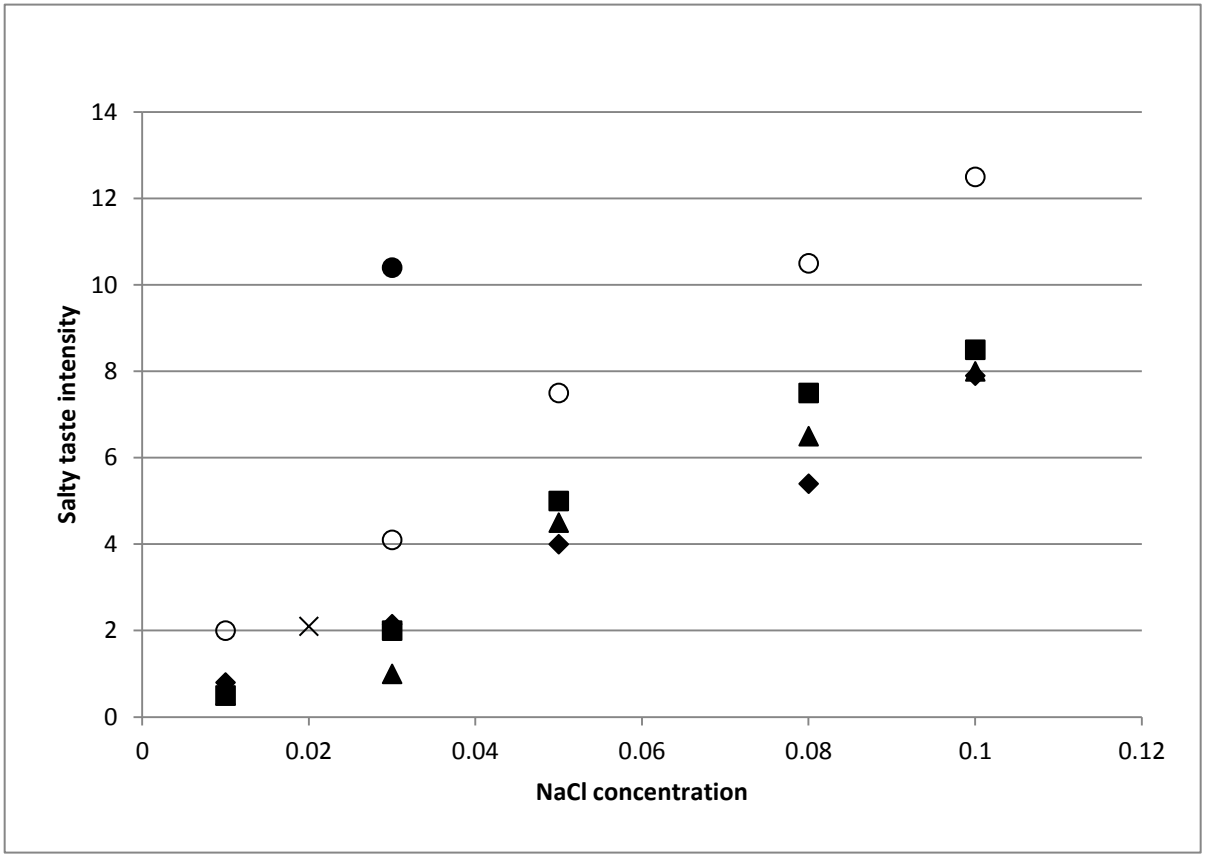

Figure 2.8 Salty taste response curves of NaCl and 3.84mM Orotic acid with and without lactose *(♦)- NaCl, (○)- NaCl and orotic acid,(■) – NaCl, orotic acid, and 5% lactose,(▲)- NaCl, orotic acid, and 10% lactose, (×)-Mean of whey permeate, (●)- mean of reduced lactose permeate, 0 to 15 point universal scale used for intensities*

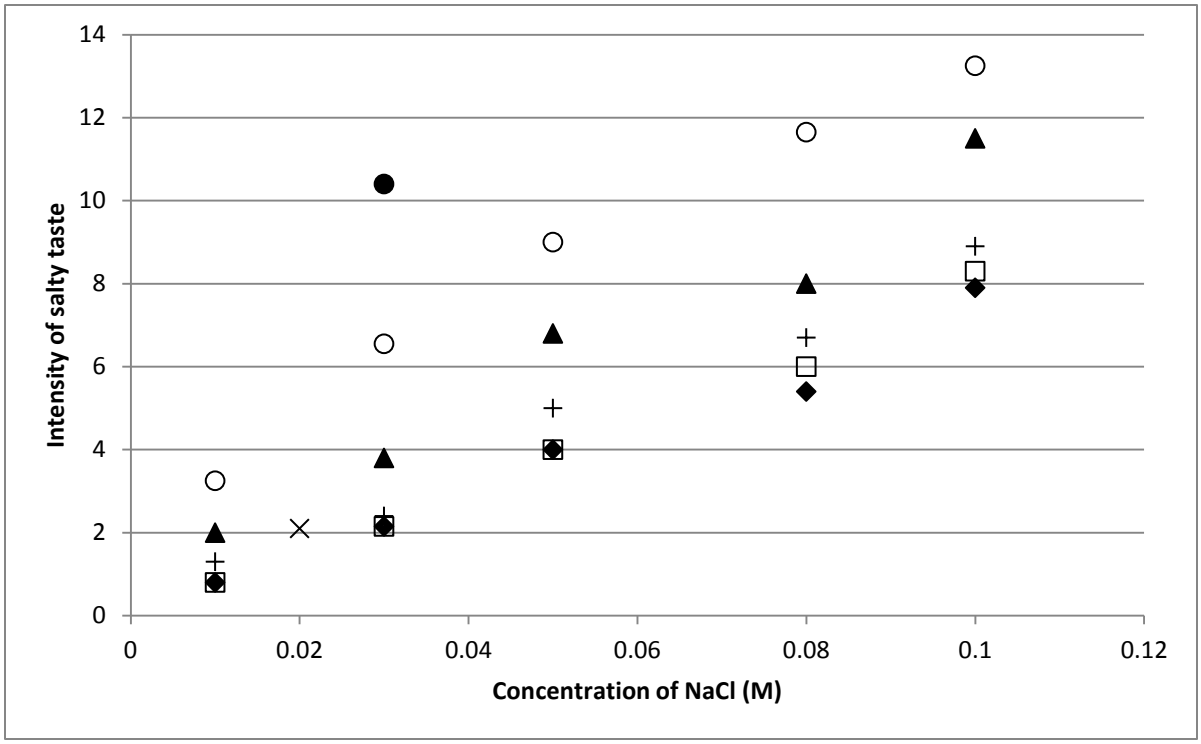

Figure 2.9 NaCl, KCl, acid, and lactose models *(♦)-NaCl and KCL with lactose,(○)- Nacl, KCl, and lactic acid with lactose,(▲)- NaCl, KCl, and orotic with lactose, (+)- Nacl, Kcl, and citric with lactose, (□)- NaCl, KCl, and hippuric with lactose (×)- Mean of whey permeate, (●)- Mean of reduced lactose permeate. 0 to 15 point universal scale used for intensities*

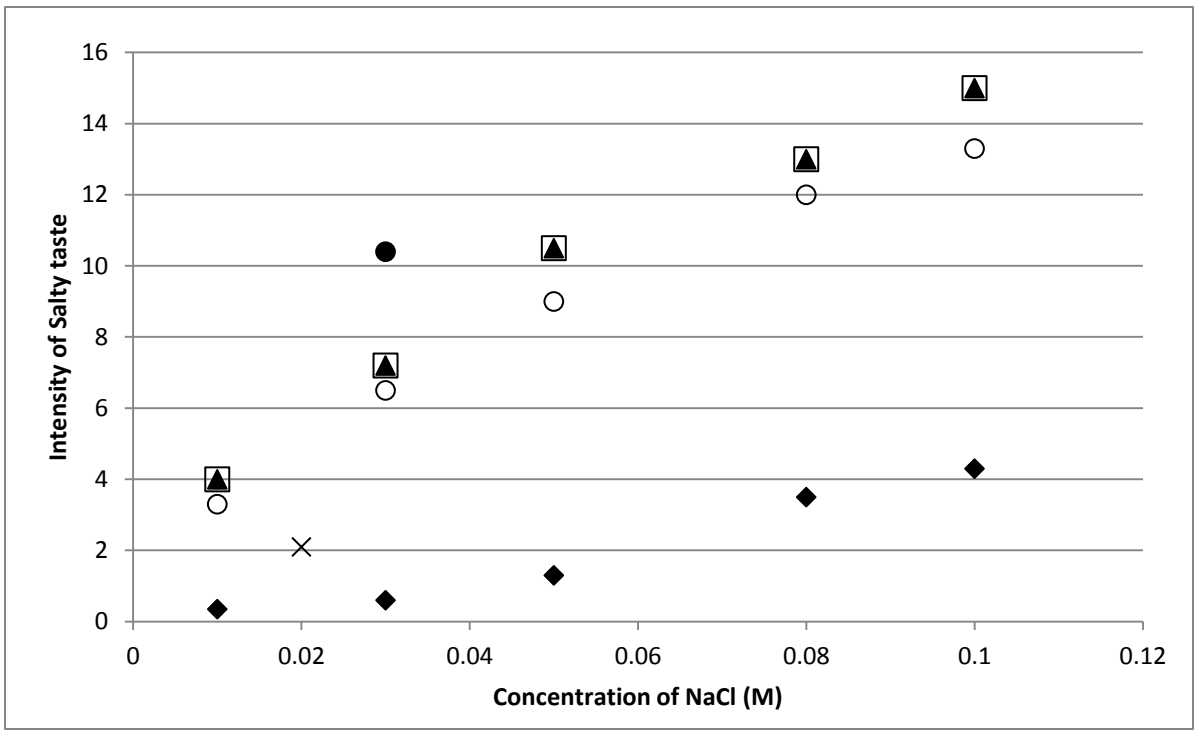

Figure 2.10 Salty taste response of different combinations of possible salty taste enhancers*(♦)- NaCl, (○)- KCl, lactic, and lactose, (□)- KCl, lactic, lactose and orotic, (▲)- KCl, lactic, lactose, citric, hippuric, orotic, uric, and urea, (×)- mean of whey permeate, (●)- mean of reduced lactose permeate. 0 to 15 point universal scale used for intensities*

APPENDICES

A1. Proximate results for milk permeate

Total solids (%)	pH	Lactose(%)
97.5	7.2	87

A2. Mineral results for milk permeate

Milk permeate	%K	%Ca	%Mg	%N	%Na	%Cl	NPN
supplier 1	1.7	11.91	1.07	1.04	1.29	0.22	2.5

A3. Organic acid concentration (ppm) for milk permeates

	Citric	Orotic	Lactic	Uric	Hippuric
S1	1551	119	458	653	20

A 4. Sensory profiles for milk permeates

Milk permeates													
Aroma	buttery	Cooked/milky	Cardboard	Potato	Beefy	Graham Cracker	vitamin	Sweet	Sour	Salty	Bitter	umami	astringent
3	3	3	ND	1	ND	ND	3.4	7.5	ND	2.1	ND	4.3	ND

A5 . Volatile compounds of milk permeate

	S1
Diacetyl	4.50a
1-hexen-3-one	3.33a
1-pentanol	2.97a
DMDS	0.03a
2-methyl-3-furanthiol	0.01a
benzaldehyde	1.27b
4-octanone	3.16b
p-cresol	0.03a
2,6-nonadienal	0.49a
DMS	3.07a
Pentanal	3.89a
Hexanal	0.93b
Methional	0.01a
Heptanal	5.82b
Nonanal	1.59a

S- supplier, different lettering within row is significantly different ($p<0.05$), values in ppb