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Evaluation of Coagulants on Soy Cheese Making Efficiency

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Abstract: The unavailability of nutritious food and the high cost of animal protein are the main causes of protein-energy malnutrition in Ethiopia. Malnutrition is predominantly seen among the rural population since the food of the population is based on crops. Promoting consumption of locally available protein-rich crops can reduce the problem of protein-energy malnutrition. This research was undertaken to investigate the effects of three coagulating agents (Lemon juice extract, Vinegar and $CaCl_2$) on the yield percentage, nutritional composition, Sensory and microbial properties of soy cheese samples produced from belesa-95 variety was studied. The percentage yield ranged from 88.64% for cheese coagulated with $CaCl_2$ to 122.23% for cheese coagulated with Lemon juice extract; and were significantly different ($p<0.05$). The cheese samples proximate compositions had varies from (53.50-58.5276% protein, 11.70442-12.93227% fat, 2.05764-3.80641% ash and 17.5826-250768% carbohydrate), on dry weight basis. Results indicated that the three coagulants significantly ($p<0.05$) modulated the various proximate parameters evaluated, The cheese coagulated by $CaCl_2$ the highest contents of protein and ash but the protein content were not significant with cheese coagulated by lemon Juice extract. Further studies revealed to sensorial attributes. The sensory acceptability of soy cheese was carried out by Melkassa Agricultural Research Center staff member and the preference test was done using nine point hedonic scales. The highest score was achieved by the lemon juice coagulated cheese was more preferred in terms of flavor, color and overall acceptability, but there were no significant differences between sensory characteristics of soy cheese coagulated by these three coagulants on overall acceptability. The Standard plate count cheese samples had reported for this work range between ($1.956* 10^3$ CFU/gram to $6.275* 10^3$ CFU/gram). Total coliform and total yeast/ mold were <10 CFU/gram, This mean Standard plate count, total coliform, mold and yeast counts showed that all of the cheese samples were microbiologically acceptable.

Key words: *Coagulants, $CaCl_2$, Lemon Juice Extract, Vinegar Solution, Soy Cheese*

I. INTRODUCTION

Protein deficiency and malnutrition is a well-documented cause of ill health and death in developing countries, and plays a major role in the disease, kwashiorkor (*Latham, M.C. 1997*). Ethiopia is one of the poorest and least developed countries in the world. The economy is based on small landholder agriculture, with more than 85% of the population of 63 million living in rural areas under very poor conditions. Ethiopia is also one of the countries in the Sub-Saharan Africa with the highest rates of malnutrition, in addition to lack of adequate and balanced diet, the increasing growing number of people who are attacked by diseases that occur due protein-energy malnutrition.

The unavailability of nutritious food and the high cost of animal protein are the main causes of protein-energy malnutrition in Ethiopia. Malnutrition is predominantly seen among the rural population since the food of the population is based on crops. Most of the dietary food in the country is supplied by cereals that are relatively poor sources of protein. Moreover, the high cost of fortified nutritious proprietary complementary foods is always beyond the reach of most Ethiopian families; hence its high malnutrition rates in Ethiopia pose a significant burden in economic and social development (*Fottrell E et al.,2009)*.Major contributing factors to malnutrition among family are poverty and low purchasing power(*Woldemariam et al,2002)*,

Protein energy Malnutrition deficiency can also lead to several ailments including mental retardation and also affect physical growth, morbidity, mortality, cognitive development, reproduction, and physical work capacity *(Mahgoub S.E.O.,et al 2006)*.

Ethiopia has a vision for national development in this new millennium. Such vision will rely on adequate human resources and will only be realized if the children being conceived and born today are given the opportunity to live to their full capacity. However, this opportunity may be outside the reach of many Ethiopian children because of malnutrition. To reduce malnutrition Food-based strategies are key to addressing hunger and malnutrition. On the other hand, attention given to locally available protein-rich crop like soybean is very poor nationally.

Soybean (*Glycine max*) is an important world commodity, due to its wider range of geographical adaptation, unique chemical composition, good

nutritional value, functional health benefits and industrial applications. The beans are generally not eaten in the fresh state but as processed product (*Osho, S.M. 1989)*. This is because; its nutritive value is improved by processing into product such as soymilk, soy flour, roasted soybean and curd *(Cowman, J.C. and Wolf, W.J. 1977).*

Soybeans have been transformed into various form of product such as soybean ayib, among soybean food one of the most important and valued soy foods product which contains high quality protein in Ethiopia.

Soybean ayib is one of the Ethiopian cottage type cheese also known as soybean curd its similar to Tofu, is made by coagulation of heated soy milk with a coagulant, followed by molding and pressing the cheese to drained away the liquid whey. Soybean ayib preparation generally includes blanching, soaking and grinding of soybeans in water, filtering, boiling and coagulation of soy milk. The yield, quality and texture of tofu are influenced by many factors such as variety of soybeans (*Shen et al., 2006*), processing methods (Shih et al., 2006) and type and concentration of coagulants (*Lim et al., 2006*).

The grouping together of small particles in a solution into larger particles, such a solution eventually coagulates with the particles forming either a precipitate or a gel. In the case of soybean ayib, coagulation of the casein protein and oil (emulsion) suspended in the boiled soy milk is the most important step in the production of soybean curd (*Jianming, W et al*.,2013).This process is occurs due to the cross-linking of protein molecules in the soymilk with the aid of coagulants. Usually, calcium sulphate and glucono-δ-lactone are used more than other coagulants on an industrial scale for the tofu making (Lim et al., 2006).

Various coagulants used in curdling or coagulating soymilk have been listed (Sri Kantha, S.,et al.,1983).The most commonly used coagulants are calcium and magnesium salts and glucono-δ-lactone depending on tofu type *(Panyathitipong, W. and Puechkamut, Y. (2008)*. It has also been reported (*Shokunbi, O.S et al., 2011*) that coagulants influence the yield and micronutrients contents of tofu additionally the effects of the coagulants on the functional properties of soybean curds have rarely been investigated (*Obiegbuna James E et al., 2014)*.

In Ethiopia, soybean is usually processed traditionally into cheese by using lemon juice as a coagulant. This is because lemon juice could serve as the cheap source of coagulant. Furthermore, lemon is readily available in the local market in all seasons. Despite wide application of lemon juice, its coagulation efficiency is not evaluated. The objective of the current study was to evaluate Nutritional; Microbial and Sensorial property of Soybean ayib making efficiency prepared from belesa-95 soybean variety and compared it with those prepared with vinegar and calcium chloride ($CaCl_2$).

II. MATERIALS AND METHODS

A. Materials and Methods

Belesa-95 variety of soybean (*Glycine max*) was collected from Pawe Agricultural Research Center, Ethiopia. The coagulants Vinegar and calcium chloride($CaCl_2.2H_2O$) food grade) was purchased from local supermarket but the lemon fruit was collected from Melkassa Agricultural Research Center, The extract of Lemon juice was prepared at Food science and Postharvest Technology Laboratory, Melkassa.

1. Preparation of coagulants and Soy cheese

The Lemon fruits was washed and cut into halves. the extracted juice of lemon was filtered through a sieve to remove the seeds. Vinegar and calcium chloride ($CaCl_2.2H_2O$) food grade) coagulants were purchased from local supermarket and food grade chemical store respectively.5% of each coagulant (lemon juice, vinegar and calcium sulfate) were added separately to each of the boiled milk with constant stirring.

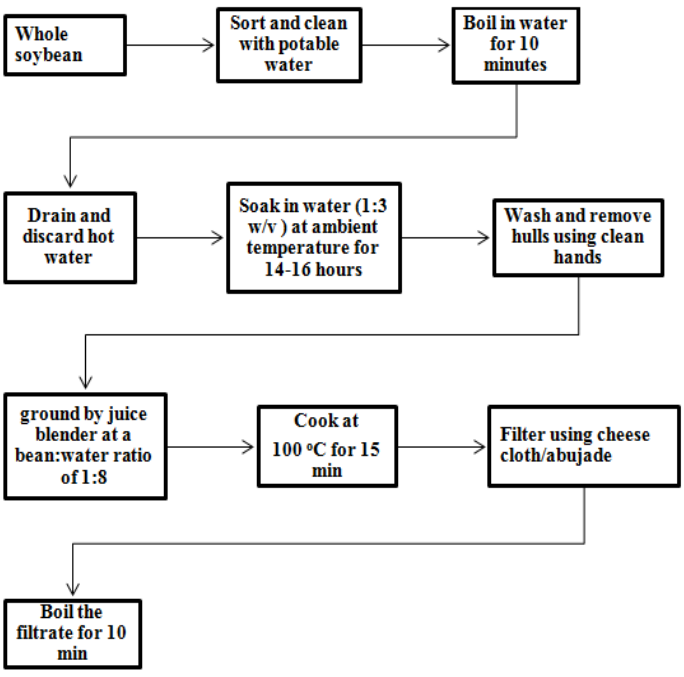

Figure 1: Soy Milk Preparation Diagram

250 gm of Belesa-95 soya bean was taken separately for each of the three coagulating agents and were soaked separately in water 1:3 w/v (three times bean weight) for 14-16 h at ambient temperature (24±2 °C). The soaked soybeans was drained, weighed and ground with a Juice

blender. The raw bean to water ratio was maintained (1:8w/v) by adding water. The mash was heated and boiled for 10 minutes and filtered through muslin cloth/abujade/ then after filtrate again heated soy milk for 15min to eliminate the cross contamination of microorganisms. The temperature of milk was lowered to 75 °C. Then the coagulating agents were added with continuous stirring to prevent sticking of solids and scorch in. The concentration of the salt (calcium chloride ($CaCl_2.2H_2O$) food grade) used was 0.5%, Vinegar was 5.0%.and the titratable acidity of lemon juice was 5.0%. The resultant curd was transferred to a home-made mould (20 x 20 x 15 cm^3) lined with muslin cloth/abujade/ and pressed for 3 h by placing a weight of 5 kg. After pressing, soya cheese samples were weighed and the produced cheese stored at 4°C for yield measurements and analysis.

B. Determination of Yield

The yield of cheese was calculated based on cheese obtained and the soybean used. The yield calculated by following ways (*Andika et al., 2011*).

Yield of fresh cheese % = (weight of fresh cheese (g)/ weight of soybean (g)) x 100%.

C. Proximate Analysis of Soy Cheese

Moisture content was determined by drying the samples at 105 ^{0}C for 3 hours. Protein content (% N x 6.25) was determined by the Kjedahl method. Crude fat test was carried out based on Soxhlet extraction method utilizing petroleum ether. The ash content was determined by dry ashing in muffle furnace at 550 ^{0}C. Dietary fiber was determined by digesting defatted samples with diluted (1.25%) sulphuric acid (H_2SO_4) solution for 30 minutes at boiling point followed by digestion with 1.25% sodium hydroxide (NaOH) solution for the same duration. All the analyses were carried out in triplicates and in dry weight basis.

D. Sensory Evaluation of Soy Cheese

Sensory acceptability of the products was evaluated by panelists based on their voluntariness to participate in the evaluation (*wunwisa k.et al, 2010)*. Thirty (30) untrained panelists, composed of males and females, scored the sensory characteristics of cheese. Sensory attributes of the soy cheese like color, taste, flavor, texture and over all acceptability were evaluated by the panelists. nine point hedonic scales (**9**-like extremely, **8**-like very much, **7**-like moderately, **6**-like slightly, **5**-neither like nor dislike, **4**-dislike slightly, **3**-dislike moderately, **2**-dislike very much and **1**-dislike extremely) was used for each attribute. Each panelist was sat in isolated place (booth) to limit any disturbances. All samples were coded and presented in a randomized arrangement.

E. Microbial analysis of Soy Cheese

Microbiological analysis was done according to *Shakir et al*. (2009). Isolation and enumeration of bacteria were done by observing growth in selective media. Cheese samples were taken aseptically and homogenized in 99 ml sterile peptone water 0.1% in a blender for about 2 minutes and serial dilutions were made. One milliliter of each dilution were pour plated in sterile Petri dishes, the stomacher dilution represent the 10^{-1} dilution, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilution prepared by using 9 ml peptone water tubes then purple red colonies on VRBD agar plates were counted as total coliform after incubation at 35 °C for 24h. The total bacteria count was enumerated on PCA plates after incubation at 35 °C for 48 hour. Yeast and mold colonies were counted on potato dextrose (PDA) agar plates after incubation at 25 °C for 5 days. Counts of visible colonies by using colony counter was made and expressed as CFU/gram of the original sample from the mean of three replicates.

F. Data Analysis

Data were statistically analyzed by the help of SAS 9.0 using one way analysis of variance (ANOVA). List significant differences were calculated at $p< 0.05$. After analysis of data, mean comparison was made for all the significant treatments and mean ± standard deviation is presented in the tables of results

III. RESULT AND DISCUSSION

A. Coagulants Response on Proximate Composition

Table 1 shows the results of nutritional composition of soy cheese for moisture content, total mineral content, crude protein, crude fat and carbohydrate. The moisture content of cheese samples varied from 68.307 % to 74.655%. The variation in the moisture content of cheese prepared with different coagulants is probably due to the differences in gel network within the cheese particles that is influenced by different anions and its ionic strengths towards the water holding capacity of soy protein gels by *V.A. Obatolu*. It may also be due to the unique coagulating properties of the coagulants used.

The ash content reported in this work (2.058 % -3.806%) is lower than the5.80–8.80%, 5.64–5.76%, 3.57-4.24% and 5.2–7.9% reported by *Shokunbi, O.S et al., Shihet al., Bhadwajet al*. and Obatolu respectively. These differences may be due to the different type of coagulants used. This trend will likely be noticed, if the mineral analysis of the samples is evaluated, cheese coagulated with $CaCl_2$ had the highest ash content, thus might be richest in micronutrients. The various modulating effects notable in the values of the proximate parameters is a reflection of the different coagulants used.

The values reported for protein (53.500–58.528%) reflects the high protein content of soybeans, which makes it useful in combating protein-energy malnutrition, especially in the rural communities of developing countries. The observed high protein content is very encouraging as protein deficiency is a serious cause of ill health and death in developing countries and plays a part in the disease kwashiorkor.

Fibers are plant based food components made of lignin, cellulose, hemicelluloses, pectin, gum and mucilage; which remain undigested on entering the human large intestine. The Fiber content reported in this work not detected thus might be in left with okara.

Table 1: Nutritional Compositions of Prepared Soya Cheese with Three Different Coagulants.

Coagulants	Nutritional compositions					
	Moisture (%)	Ash (%,d.b.)	Fat (%, d.b.)	Protein (%,d.b.)	CHO (% d.b.)	Fiber (% d.b.)
Lemon Juice extract	74.655^{a} ± 0.173	2.69935^{b} ± 0.055	11.70442^{c} ± 0.341	57.0487^{a} ± 0.385	57.0487^{a} ± 0.385	ND
Vinegar	72.196^{b} ± 0.052	2.05764^{c} ± 0.011	12.93227^{a} ± 0.128	53.5 00^{b} ± 0.601	53.5 00^{b} ± 0.601	ND
Calcium chloride solution	68.307^{c} ± 0.041	3.80641^{a} ± 0.018	12.34999^{b} ± 0.033	58.5276^{a} ± 1.015	58.5276^{a} ± 1.015	ND
CV	1.543	1.467	0.490	1.753	1.64	-

Values are averages of triplicate readings (mean ± standard deviation) Where, CV= coefficient of variance, CHO=carbohydrate. Means followed by different superscript letter within the column indicate significant difference at ($P < 0.05$).

B. Coagulants Response on Yield Percentage

Table 2 shows the results of yield percentage of soy cheese treated with lemon juice extract, Vinegar and calcium chloride solution. The result of cheese percentage were varied from 88.640% to 122.213 % Calcium chloride cheese had the least yield and was significantly lower than the yield of Lemon juice and vinegar cheese. The yield and quality of tofu have been reported to be influenced by soybean varieties, soybean quality, processing conditions and coagulants. The yields of cheese depend on different factors, *Hou et al.(1997)*, reported that the coagulants, stirring speed and stirring time had a significant influence on yield and textural properties of tofu. Hou and Chang (2003) reported that storage time also influences the yield of cheese. The yield of cheese was also found to be influenced by heating time *(Noh et al., 2005).*

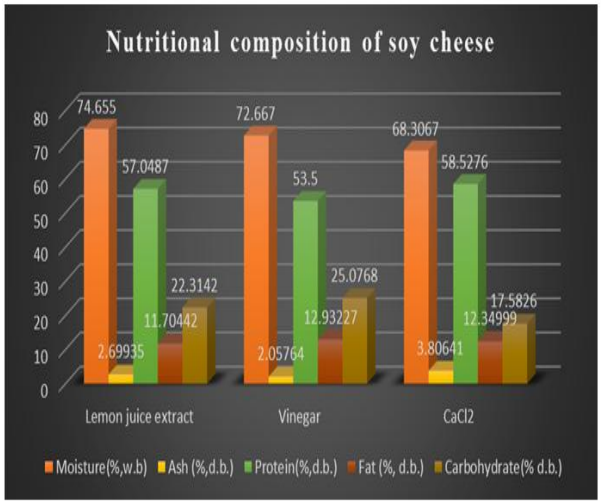

Table 2: Percentage Yield of Cheese Processed with Different Coagulants

Coagulants	Yield (%)
Lemon Juice extract	122.213 ± 1.575^{a}
vinegar	108.947 ± 3.628^{b}
Calcium chloride solution	88.640 ± 1.569^{c}

Values are averages of triplicate readings (mean ± standard deviation). Means followed by different superscript letter within the column indicate significant difference at ($P < 0.05$).

C. Coagulants Response on Sensory Acceptance

Table 3 shows the results of sensory evaluation of soy cheese for color, flavor, taste, texture and overall acceptance. The results were expressed on a 9-point hedonic scale there were no significant differences between sensory characteristics of soy cheese coagulated except color and texture. This means that coagulants had little effect on the sensory attributes of soy cheese.

Table 3: Effect of Coagulants on Sensory Characteristics of Soy Cheese.

Coagulants	Color	Flavor	Taste	Texture	Overall acceptability
Lemon Juice	7.233± 0.058^{a}	7.100± 0.458^{a}	6.400± 0.436^{a}	6.333± 0.503^{b}	6.867± 0.252^{a}

extract					
vinegar	6.433± 0.208[b]	6.300± 0.361[a]	6.533± 0.321[a]	6.533± 0.252[ab]	6.633± 0.306[a]
Calcium chloride solution	6.667± 0.493[ab]	6.367± 1.060[a]	6.033± 0.153[a]	7.400± 0.520[b]	6.600± 0.300[a]

Values are means of triplicates ± standard deviation, the means with the same letter are not significantly different at 95% Confidence Interval (P<0.05).

D. Coagulants Response on Microbial Safety

Table 4 shows the results of microbial load of soy cheese for total bacteria count, total coliform count and yeast/mold count were evaluated. The total bacteria count reported in this work ranged from 1.956* 10^3 CFU/gram to 6.275* 10^3 CFU/gram, the total coliform and mold/ yeast count were absent or <10 CFU/gm. From the microbiological point of view the product is acceptable in quality because no significant amount of microorganisms were found in the cheese; these values are in agreement with that reported by (*Nazim et al., 2013).*

Table 3: Effect of Coagulants on Microbial Load of Soy Cheese

Coagulants	Total bacteria count CFU/g	Coliform count CFU/g	Yeast and Mold count CFU/g
Lemon Juice extract	$2.567*10^3 \pm 6.110*10^2$	$\leq 1*10^1$	$\leq 1*10^1$
vinegar	$4.567*10^3 \pm 8.327*10^2$	$\leq 1*10^1$	$\leq 1*10^1$
Calcium chloride solution	$6.067*10^3 \pm 2.082*10^2$	$\leq 1*10^1$	$\leq 1*10^1$

Values are means of triplicates ± standard deviation.

CONCLUSION

This study has been confirmed the feasibility of replacing industrial vinegar solution and calcium chloride salt by extract of lemon juice for the coagulation of soybean milk in the preparation of soy cheese from belesa-95 variety, cheese coagulated by lemon Juice extract; the yield also higher, and sensory properties were better from microbial aspect safe. The cost of cheese preparation is reduced because of the cheaper price of lemon extract than that of calcium chloride and vinegar. In this way, it was concluded that the extract of Lemon Juice coagulants could be used as a natural coagulant in coagulation processes of soymilk.

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