DEVELOPMENT OF SAFE AND SUSTAINABLE BIORUBBER AND BIOLATEX FROM ANNUAL PLANT *TARAXACUM KOK-SAGHYZ* (TKS) IN CANADA

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ABSTRACT

NovaBioRubber Green Technologies Inc (NovaBioRubber) develops "green" innovative processing and growing technologies for the production of alternative biolatex, biorubber and inulin from annual rubber plant *Taraxacum kok-saghyz* (TKS) to solve latex allergy and to satisfy the growing global rubber demand.

The goal of any processing technology is to extract biorubber as pure as possible at lower cost without altering quality. Some processes cause significant physical and chemical changes in the quality of biorubber. Production of high quality biorubber at lower cost is critical in order to produce affordable biorubber for various applications. Also, environmental impact of these technologies should be carefully evaluated before choosing a process for commercial activities. NovaBioRubber has already developed and patented a "green" extraction process and proprietary processing equipment for biorubber extraction from rubber-bearing plants. Third party evaluation results are available.

Currently, Nova-BioRubber is conducting the field trials to grow TKS on various farms in British Columbia, Canada. Rubber content in TKS plants are reaching 17% after growing just 4 months. Biolatex from TKS have been tested extensively and confirmed to be safe due to lowest amounts of antigenic proteins similar to romaine lettuces. Biolatex from TKS can be used to produce non-allergenic rubber gloves. It is expected that a sustainable biorubber industry can be created based on TKS. This paper reviews growing specifics, processes stages, economics and environmental impact for the most promising processing technologies.

BACKGROUND

Annual rubber plant, *Taraxacum kok-saghyz* (TKS) also known as Russian Dandelion (aka Kazakh dandelion) was discovered in 1929 in Central Asia during the efforts of Soviet Union to gain independence for the rubber supplies. The contents of biorubber and inulin in TKS can reach 24% and 40%, respectively. About 50% of biorubber can be extracted as biolatex in liquid form.

TKS was grown extensively in Former Soviet Union, USA and Canada in 1930 and 1940s during rubber crisis (Makogon, 1936, Duff et al., 1943, Whaley and Bowen, 1947). Total area of land used for cultivation of TKS in 1940s was about 100,000 ha in Former Soviet Union alone. Recently, TKS has received significant interest from tire industry due to the higher quality of biorubber for tire applications. Price volatility of natural rubber from Hevea tree has also contributed to the renewed interest into the research on TKS.

Several processes for production of biorubber from TKS were developed since 1930s. The most promising technologies are: 1) Wet-milling 2) Solvent-based extraction and 3) Dry-milling. The goal is to extract biorubber from the root tissue as pure as possible without altering its quality at lower production cost. Early processes from 1940s were based on wet-milling in the presence of alkali and acids. High processing cost of water-milling technology is the main concern. Processing cost can reach $14.7/kg. Current price for natural rubber from Hevea tree is about $2/kg. Also, the environmental impact raises a lot of questions.

Chemical solvent-based extraction process was known since 1900s. Solvent-based process was never used for industrial processing due to the environmental concerns and human health issues. In spite of environmental concerns, there is a renewed interest in solvent-based process from tire industry due to the higher quality of the rubber produced (Randall et al., 2013).

Recently green dry-milling process was developed by our team (Buranov, 2009 and 2013). Dry-milling process is environmentally-friendly and cost-effective due to the use of unique combination of mechanical forces in dry medium. Dry-milling offers a lot of cost savings in processing costs such as energy, labor, chemicals and water consumption compared to other processes. The processing cost can be as low as $1/kg. The demonstration of green dry-milling process in industrial settings is under development. Environmental benefits of dry-milling are numerous. Dry-milling process and proprietary machinery have been been validated by the third party National Research Council Canada in 2015. The evaluation reports are available.

The focus of this study is to review the growing practices and main steps of all three processes considering economic and environmental factors.

FIELD TRIALS OF TKS IN CANADA

Growing activities were initially started in a greenhouse in Abbotsford, British Columbia, Canada and moved to the farms in 2015. Plastic containers and raised beds filled with commercial soil were ideal for greenhouse growing and seed production activities (Fig 1 and 2). Field trials on various farms indicated that climate in British Columbia is ideal for growing TKS plants.

TKS plants grow well between +10+25 ^{o}C. Prolonged high temperatures have deleterious effects on TKS. Rainfall must be at least 381 mm during growing season. TKS doesn't survive under -6.6 ^{o}C. (Whaley and Bowen, 1947)

Climate of British Columbia were found ideal for growing TKS plants. No summer dormancy was observed in British Columbia due to proximity of Pacific Ocean that has cooling effect and bring about abundant rainfall up to 360-560 mm during May-August. Summer dormancy of TKS plants are observed in dry and hot climates. Field trials at Ohio State University and Guelph University confirmed that summer dormancy occur between July-August due to dry and hot climates (private communications). TKS plants do not survive in Ohio and Ontario in winter due to the cold temperatures. During the winter, the temperature in British Columbia is about +1-3 ^{o}C which are ideal for plants to stay in the ground without freezing.

Figure 1. Greenhouse growing activities in Abbotsford, BC, Canada in 2014

Figure 2. Field trials in Abbotsford, BC, Canada in 2016

TKS plants grow slowly during the first 30 days of germination. This is due to the fact of root development. Weed control is necessary to achieve higher yields. Commercial herbicides are widely available to control weeds.
Rubber contents in plants in TKS plants reach up to 17.5% after 4 months of growing (Table 1). Biorubber accumulation happen mostly in cooler moths of September-November.

Table 1. Rubber contents (in %) in TKS plants

Species	July	August	September	October	November
Greenhouse plants					
1	4	8.1	13.8	14.2	14.8
2	4.5	8.5	12.7	12.9	13.8
3	4.3	9.2	14.7	15	15.7
Field crops					
1	3.1	5.6	10.2	15.4	17.5
2	2.5	5.7	9.3	13.4	15.5
3	1.2	3.2	8.4	11.1	12

The yield of TKS roots can reach up to 20 tons of fresh roots per hectare. The yield of biorubber can be up to 1500 kg/ha through dense growing. All growing activities can be mechanized and therefore, labor cost will be minimal.
British Columbia is ideal to grow TKS plant due to the cooler temperatures compared to other provinces and the USA. Therefore, it is important to establish a sustainable biorubber industry in British Columbia, Canada.

WET-MILLING PROCESS

Wet-milling process was developed in 1940s by Roderick Eskew and his team (Eskew, 1946; Stamberger, 1946). Wet-milling process is based on the crushing of plant tissue via extensive pebble milling in hot water and agglomerating rubber as “worms”. The rubber worms still contain significant amounts of residual root tissue and must be purified. Rubber worms are purified by boiling in hot alkali solution to dissolve residual plant tissue. The rubber quality is significantly deteriorated due to the high temperature, chemicals and extensive milling.

Process description

Wet-milling process is the complex multi-step process. Processing of TKS to produce rubber consists of two major stages: 1) Rubber recovery and 2) Rubber purification. Each stage has major 5 steps or unit operations.

Rubber recovery process includes the following 5 steps: 1) extraction of inulin, 2) pebble-milling 3) screening 4) pebble-milling again 5) flotation.

First, the roots (dried or fresh) are leached in hot water to extract inulin. Then, the remaining root tissue is extensively pebble-milled in hot water (80-100 ^{o}C) to crush the root tissue and agglomerate the rubber. It is further screened to remove the fleshy part of the roots from the mixture of rubber and roots skins. The mixture of rubber and skins are again pebble-milled with hot water to completely disengage the rubber from the skins. Rubber is separated from the skin via flotation. Flotation is carried out at hydrostatic pressure of about 250 psi at a temperature of 93 ^{o}C. Then, the rubber floats and is skimmed off. The rubber still contains 10-15% impurities mostly root tissue and therefore, it was called “contaminated rubber”.

Purification of the contaminated rubber also consists of five major steps or unit operations: 1) boiling the contaminated rubber in hot alkali solution 2) flotation of rubber 3) scrubbing the alkaline-treated rubber with water-soluble fatty acid 4) washing the rubber and 5) drying the rubber.

Purification of contaminated rubber is not easy process due to the complete incorporation of plant tissue in the rubber mass during pebble-milling. In order to efficiently purify the rubber mass, flaking into thin slices are necessary to make more accessible to the alkaline solution. The contaminated rubber is boiled in hot alkali solution such as sodium hydroxide at 100 ^{o}C. Dilute solution of sodium hydroxide (1-5%) can be used. More concentrated (20%) can be used to reduce the time of boiling to 30 min. The impurities in the contaminated rubber can be reduced significantly due to the degradation of residual root tissue in hot alkaline solution. Dark toxic waste solution is generated as the result. Further, the rubber is separated from the toxic waste using flotation at 250 psi. The rubber is further purified from root tissue by scrubbing at ~80 ^{o}C in the solution of fatty acid (such as stearic and palmitic acids) as well to completely remove caustic solution via neutralization. The resulting rubber is washed and centrifuged to remove access water and dried under vacuum.

The quality of this rubber significantly deteriorates during high temperature and caustic treatment. This process produces a lot of toxic alkaline and acidic wastes as well.

The first stage, rubber recovery stage of wet-milling process was demonstrated on a pilot-scale rubber production facility with production capacity of 10 tons/day in the USA in 1940s (Whaley and Bowen, 1947). The process scheme for rubber recovery stage is illustrated in Fig. 3. The second stage, rubber purification stage is not shown. Pilot-scale processing factory is much more complex than original patents and involves many steps to produce rubber (Fig. 3.) The pilot-scale processing starts with the drying of the roots on battery driers. Then the roots are bailed and stored until processed. Inulin is extracted in boiling hot water and the syrup is stored in holding tanks. Then the roots are pebble milled and screened two times to remove the bagasse. Further, rubber is separated from the root skins via flotation and screened before sending to centrifuge. After the centrifugation, anti-oxidant is added and the rubber is dried. Rubber purification stage is not described in this scheme.

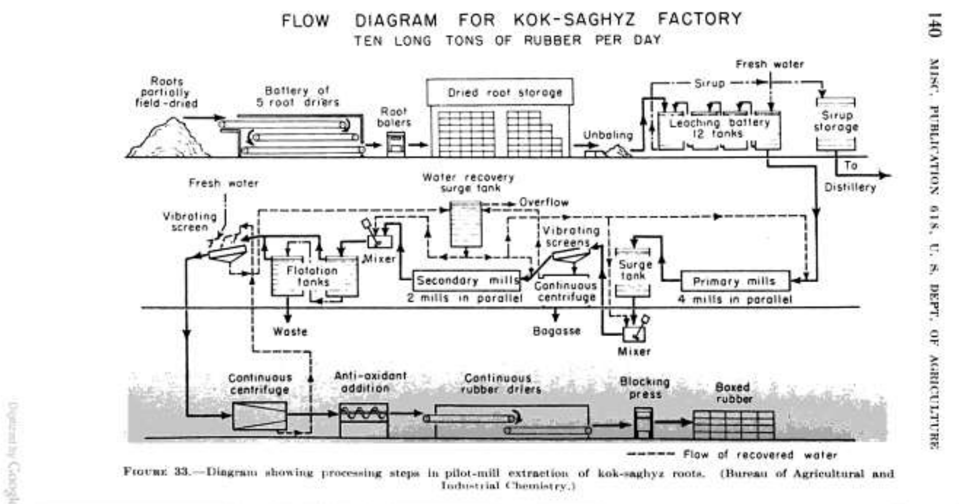

FIGURE 33.—Diagram showing processing steps in pilot-mill extraction of kok-saghyz roots. (Bureau of Agricultural and Industrial Chemistry.)

Figure 3. Flowchart of water-based milling (Whaley and Bowen, 1947)

Process economics and environmental concerns.

Wet-milling process requires a lot of labor, energy, chemicals and water generating huge volumes of dark toxic wastes. This makes this process most expensive and polluting process to produce biorubber. The processing cost for the first stage was $0.44/kg of rubber in 1940s and it is now estimated at $7.35/kg of rubber considering the inflation rate of 16.7 folds over the last 70 years (see US inflation calculator). The processing cost for the second stage, rubber purification stage was not reported by Whaley and Bowen, 1947 and it can be estimated the same with the first stage. Therefore, the processing cost with wet-milling process is $14.7/kg of rubber in 2015.

Table 2. Production cost for wet-milling process in the USA and Canada in 2015.

Costs	Expenses in USA ($/kg of rubber)	Expenses in Canada ($/kg of rubber)
First stage: Rubber recovery	7.35	7.35
Second stage: Rubber purification	7.35	7.35
Growing cost	2.86	0.5
Total production cost	**17.56**	**15.2**

Growing cost in the USA was estimated at $208/acre in 1940s due to the use of manual labor for weeding (Whaley and Bowen, 1947). The current growing cost is estimated to be around $400/acre in 2015 and the yield of rubber is expected to be $300 pounds/acre in the USA due to the warmer climates. The growing cost per pound of rubber is $1.3 per pound (~2.86/kg of rubber). According to our preliminary results, the growing cost in Canada is expected to be $0.5/kg lower due to the cooler temperature that eliminates the need for extensive irrigation and increases the rubber yield. Land cost in Canada is significantly lower than in the USA.

Total production cost of rubber is expected to be around $17.56/kg in the USA and $15.2 in Canada. For comparison, the current rubber price is ~ $2/kg of rubber and it reached maximum of $5.6kg in 2011. Therefore, commercial viability of wet-milling process is unlikely compared to the current rubber prices even though inulin is produced as a byproduct. The environmental concerns also raise a lot questions about viability of this process. Building waste treatment facility and recycling the dark toxic wastes contributes to the production cost of rubber negatively.

SOLVENT-BASED PROCESS

Non-polar solvents such as toluene and hexane can dissolve biorubber. Solubility of biorubber is only ~2% due to the higher molecular weight (~1.7 million) of biorubber (Schloman, 1988; Schloman, 2005; Buranov, 2005). Toluene is slightly better solvent than hexane based on solubility parameters. However, the carcinogenicity of toluene raises a lot of health risk questions. Lower solubility requires the use of large volumes of solvents and extensive recycling that consumes a lot of energy. Solvent loss and explosiveness of organic solvents pose serious threat against the health of local people and surrounding areas.
The fundamental problem in solvent extraction of rubber from plant materials is that rubber is a high molecular weight polymer unable to pass cell walls and membranous tissue in solution. This results in impractically slow extraction and very large solvent losses in pilot-plant operations (Kay and Gutierrez, 1987; Buranov 2009).

Process description

The use of solvents in the processing of rubber-containing plants has been known since 1900s (Prokofiev, 1936; Schloman, 1988; Schloman, 2005; Kay and Gutierrez, 1987; Buranov, 2005). Solvent-based technology was mainly used for processing guayule shrub (Schloman, 1988, 2005, 2010; Kay and Gutierrez, 1987; Beinor and Cole, 1986).

Solvent-based process consists of two main stages: 1) Simultaneous rubber and resin extraction and 2) Rubber precipitation.

Overall, there are 10 major unit operations (or steps): 1) drying the shrub 2) grinding and flaking the shrub 3) simultaneous rubber and resin extraction 4) centrifugation 5) rubber precipitation 6) decontamination of rubber 7) distillation of solvents (or azeotropes) 8) desolventization of rubber 9) desolventization of resin 10) desolventization of the bagasse.

The flowchart of the solvent-based process is illustrated in Fig.4 (Foster, 1991). Shrubs should be completely dry in order to do solvent extraction. Efficiency of solvents drops significantly if the shrubs are not dried properly. Therefore, the shrubs are dried thoroughly. Grinding and flaking make the plant tissue more accessible to solvents. The simultaneous extraction of rubber and resin is carried out simultaneously with a mixture of non-polar polar solvent (hexane or pentane) and smaller amount of polar solvent (acetone or ethanol). Usually the mixture of hexane (or pentane) and acetone is used with the ratio of 80:20 (Schloman 2010). The extraction of rubber is very slow due to the higher molecular weight rubber to pass through the cell walls and membranous tissue in solution. The resident time of solvent is usually 24 hours and requires multiple extractions (Schloman, 2005; Buranov, 2005). The slow extraction makes the process commercially impractical and results in significant solvent losses. Further, the impurities are separated from the rubber solution using continuous centrifuges. The rubber is precipitated by adding 3-4 volumes of acetone. This is the significant volume of acetone that must be recycled. The rubber is decontaminated from resin with the fresh volumes of hexane or pentane. Separation of azeotropes requires extra efforts and energy costs. Further, rubber is desolventized. Resin and bagasse are also desolventized to recover solvent and to reduce the environmental impact. Solvents are recovered for reuse. However, the solvent loss is unavoidable and this is significant risk to the environment and human health. Solvent loss also poses explosion risk.

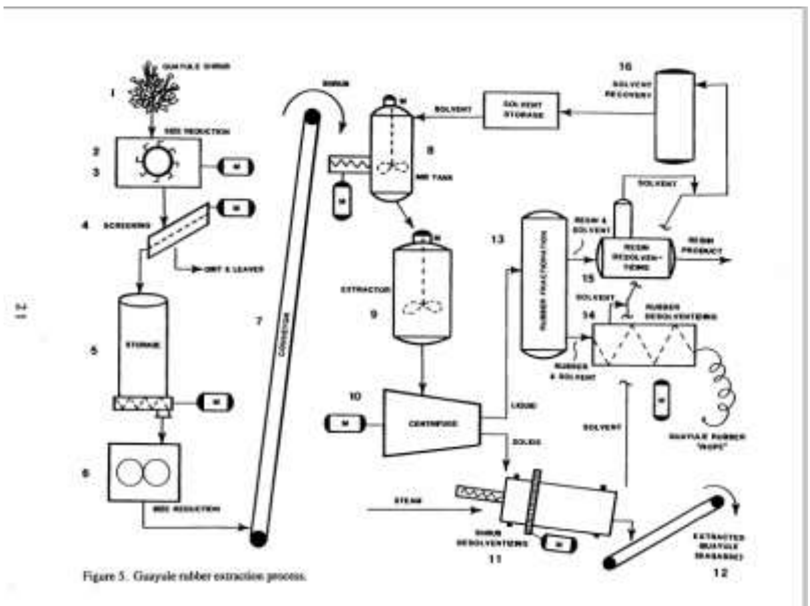

Figure 4. Guayule rubber extraction process (Foster et al., 1991)

In 1986, a pilot-scale solvent-based processing facility was designed and constructed in Sacaton, Arizona by Firestone Tire & Rubber Co under financial support from US Department of Agriculture. Overall, 8.8 tons of rubber was manufactured by 1990 and the operation was ceased in the same year (Schloman, 2010).

DRY-MILLING PROCESS

Dry-milling process was developed by our team in 2006 (Buranov, 2009). The rubber is extracted using unique combination of physical forces such as gravity, rotational and frictional. No water or chemicals used. The process is continuous and carried out at room temperature on a device called rubber extractor (Buranov, 2013). Patented green extraction process, proprietary equipment, biorubber, biolatex and TKS plants were tested independently by National Research Council Canada for 8 months. Third party evaluation reports are available.

Process description

Dry-milling technology has also two main stages: 1) Rubber recovery and 2) Rubber purification.

Rubber recovery is completely dry and continuous stage. It is carried out at room temperature on a machine called rubber extractor. No chemicals, no solvents nor water used. The dry roots are fed on hopper of the rubber extractor continuously. The mixture of ground root tissue and rubber threads are produced. The mixture is sieved to separate rubber threads from ground roots. The rubber threads contain 10-15% root tissue and should be purified.

Rubber purification stage is carried out in small amount of warm water in the presence of additives. Purification of rubber threads is easier than the rubber worms produced by wet-milling process (section 2.1.) since the surface of rubber threads are more accessible to the purifying solution. Rubber threads can be easily purified using various extraction additives which can be recycled.

Currently, industrial design for dry-milling process is under development by our team in Canada.

Process economics and environmental benefits

Dry-milling process provides significant savings in processing costs in terms of energy (50%), labour (80%), chemicals (100%), water consumption (90%) compared to wet-milling process. No toxic chemicals are consumed and therefore, no wastewater is generated. As the result, no pollution is observed nor is greenhouse gas emitted. No need for recycling the wastewater saving a lot of investment into building waste treatment facility and its operation.
Dry-milling process is green and cost-effective to operate. Processing cost is expected to be around $1/kg of rubber.
Quality of biorubber is high since all process activities are carried out at room temperature and no caustic chemicals are used. TKS growing cost in Canada is expected to be the lowest ($0.5/kg of rubber) due to the ideal cooler temperature and lower land costs.

COMPARISON OF PROMISING TECHNOLOGIES

Comparison of three processes is brought in Table 3. Solvent-based process ($82 million) is the most investment-intensive process due to the need for the explosion-proof processing facility.

Growing cost of TKS in Canada is very cost-effective ($0.5/kg) especially due to the cooler ideal climate, higher rubber yields, lower land costs and less irrigation needs.

Dry-milling process has the lowest processing cost due to the significant savings in energy, labor, chemicals and water consumption. Most expensive process to operate is wet-milling process ($14.7/kg).

Growing TKS in Canada and processing with dry-milling technology will be the most profitable business venture.

Table 3. Comparison of the promising technologies for the production of natural rubber

Cost	Wet-milling	Solvent-based	Dry-milling
Investment for processing ($ million)	12.6	82	10
Growing cost ($/kg of rubber)	2.86*	6.55&	0.5#
Processing cost ($/kg of rubber)	$14.7	0.93	$ 1.0
Credit for byproducts	-$5 (inulin)	-$1.3 (resin)	-$5 (inulin)
Production cost ($/kg of rubber)	$12.56	$6.18	-$3.5
Risks & benefits	Expensive and Toxic wastes	Toxic and air pollutant. Explosive. Health issues	Green and cost-effective

*-TKS growing in the USA.

#-TKS growing cost in Canada

&- Guayule growing cost in the USA (Foster, 1991).

It is important to start commercial growing activities in Canada due to lower growing costs due to the ideal climate for growing TKS. Another reason is the affordable land costs and higher rubber yields at cooler temperatures.

ANTIGENIC PROTEIN IN BIOLATEX FOR MANUFACTURING RUBBER GLOVES

It is expected TKS biolatex will be a potential alternative to produce hypoallergenic rubber products due to its lower antigenic protein content and different proteomic compositions. The research results from National Research Council Canada showed that TKS latex proteins exhibited significantly reduced antibody affinity relative to *Hevea* latex proteins (Dafoe et al., 2017). The low antigenicity relative to *Hevea* latex proteins required slight modifications to the standardized analytical procedures in order to obtain quantifiable results, and yet the levels of antigenicity produced by TKS latex proteins remained below the recommended range for accurate quantification in both polyclonal and monoclonal quantitative ELISA assays. Visual confirmation of dramatically decreased relative antigenicity was established by Western blots probed using the same antibodies that were used for the ELISA assays. TKS is similar to romaine lettuces in terms of the content of antigenic protein (Figure 5). Therefore, TKS biolatex is supposed to be hypoallergenic.

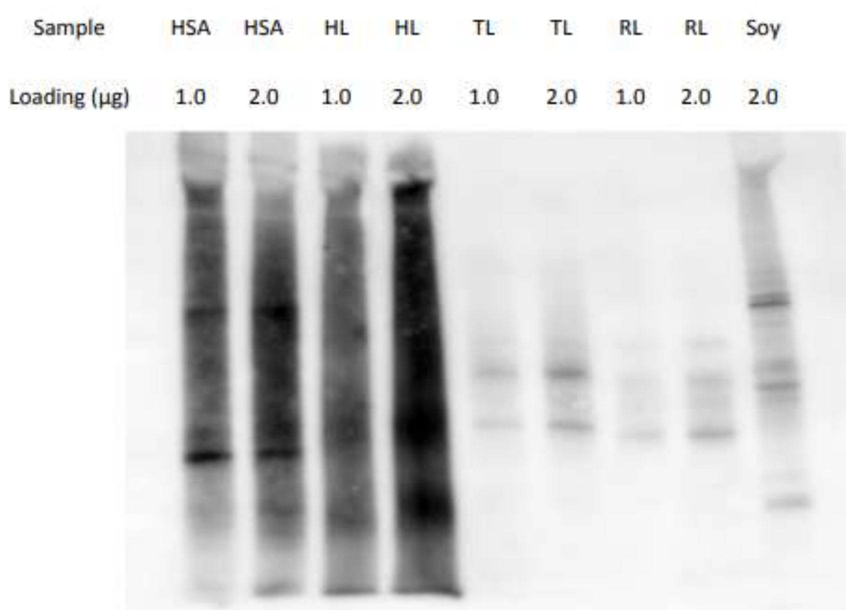

Figure 5: Polyclonal Western blot image showing antibody recognition of Hevea standard antigen (HSA), Hevea latex protein extract (HL), T. kok-saghyz latex protein extract (TL), L. sativa latex protein extract (romaine lettuce, RL) and G. max protein extract (soybean, Soy). Protein loadings (μg) are specified for each lane (Dafoe et all, 2017).

Since the test results is based solely on *in vitro* assays not *in vivo* testing with animals or susceptible individuals, it may be early to conclude TKS latex can effectively work as a substitute *Hevea* latex on the basis of hypoallergenicity. However, the significantly reduced antigenic protein content and reactivity suggest great potential for such applications. With

washing steps common in production processes, further reducing the protein content, applications for TKS-derived hypoallergenic latex may be possible.
In summary, this investigation has confirmed that the degree of antibody recognition of TKS latex proteins is much lower than for Hevea latex proteins. However, the risk for allergic reactivity toward TKS latex proteins in sensitized individuals needs to be confirmed by *in vivo* experiments.

CONCLUSIONS

TKS can be grown in British Columbia efficiently at lower cost due to the cooler climate and abundant rainfall.
Biorubber from TKS needs be extracted as pure as possible without altering rubber quality at lower production cost. Quality of biorubber is deteriorated with the effect of high temperature, residual root tissue and caustic chemicals. The potential process should not pose any risk to the environment and human health.

The wet-milling process is disadvantageous due to the excess use of labor, water, energy and chemicals. Quality of natural rubber is significantly deteriorated due to the use of caustic sodium hydroxide during the purification of rubber. The resulting dark toxic wastes pose significant risk to the environment and human health. The production cost can reach up to $17.56/kg of rubber.

Solvent-based process is also disadvantageous because of impractically slow extraction, very large solvent losses, human health issues, environmental concerns and the highest risk of explosion. Significant investment (~$82 million) is required to build an explosion-proof pilot-scale solvent-based processing facility. However, the highest quality of natural rubber is produced using solvent-based process.

Dry-milling process can be the most promising in terms of both lower processing costs and environmental benefits. Dry-milling provides significant savings in processing costs in terms of energy, labor, chemicals and water. However, the quality of biorubber must be evaluated by industry for various applications. Preliminary test results look promising. Processing cost is expected to be around $1/kg. Growing cost of natural rubber in Canada is expected to be the lowest due to cooler climate and lower land costs.

Non-allergenic rubber gloves can be manufactured from biorubber and biolatex and marketed to high value rubber glove applications.

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